ISOLATION AND IDENTIFICATION OF NEW DELHI METALLO-BETA LACTAMASE-1 GENE PRODUCING *ENTEROBACTERIACEAE* AMONG CLINICAL ISOLATES IN TIRUNELVELI

MEDICAL COLLEGE

DISSERTATION SUBMITTED TO

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE IN MICROBIOLOGY

(Branch IV) M. D. (MICROBIOLOGY)

of

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CHENNAI- 600032

DEPARTMENT OF MICROBIOLOGY

TIRUNELVELI MEDICAL COLLEGE

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"ISOLATION AND IDENTIFICATION OF NEW DELHI METALLO-BETA LACTAMASE-1 GENE PRODUCING *ENTEROBACTERIACEAE* AMONG CLINICAL ISOLATES IN TIRUNELVELI"** submitted by **Dr.R.SHINY** to The Tamilnadu Dr. M.G.R Medical University, Chennai, in partial fulfillment of the requirement for the award of M.D. Degree Branch – IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

Head of the Department,
Department of Microbiology
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CERTIFICATE

This is to certify that the Dissertation **"ISOLATION AND IDENTIFICATION OF NEW DELHI METALLO-BETA LACTAMASE-1 GENE PRODUCING *ENTEROBACTERIACEAE* AMONG CLINICAL ISOLATES IN TIRUNELVELI"** presented herein by **Dr.R.SHINY** is an original work done in the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D.(Branch IV) Microbiology under my guidance and supervision during the academic period of 2014-2017.

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Tirunelveli Medical College,
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DECLARATION

I, **Dr.R.SHINY** declare that, I carried out this work on **"ISOLATION AND IDENTIFICATION OF NEW DELHI METALLO-BETA LACTAMASE-1 GENE PRODUCING *ENTEROBACTERIACEAE* AMONG CLINICAL ISOLATES IN TIRUNELVELI**" at the Department of Microbiology, Tirunelveli Medical College, I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, or diploma to any other University, Board, either in India or abroad.

This is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D Degree examination in Microbiology.

Place: Tirunelveli **Dr.R.Shiny**

Date:

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PROTOCOL TITLE: ISOLATION AND IDENTIFICATION OF NEW DELHI METALLO-BETA LACTAMASE-1 GENE(NDM-1) PRODUCING ENTEROBACTERIACEAE AMONG CLINICAL ISOLATES IN TIRUNELVELI MEDICAL COLLEGE

PRINCIPAL INVESTIGATOR: DR.R.SHINY, MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR POST GRADUATE IN MICROBIOLOGY
DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE , TIRUNELVELI

Dear , Dr.R.Shiny, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.12.15.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
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STANDS APPROVED UNDER SEAL

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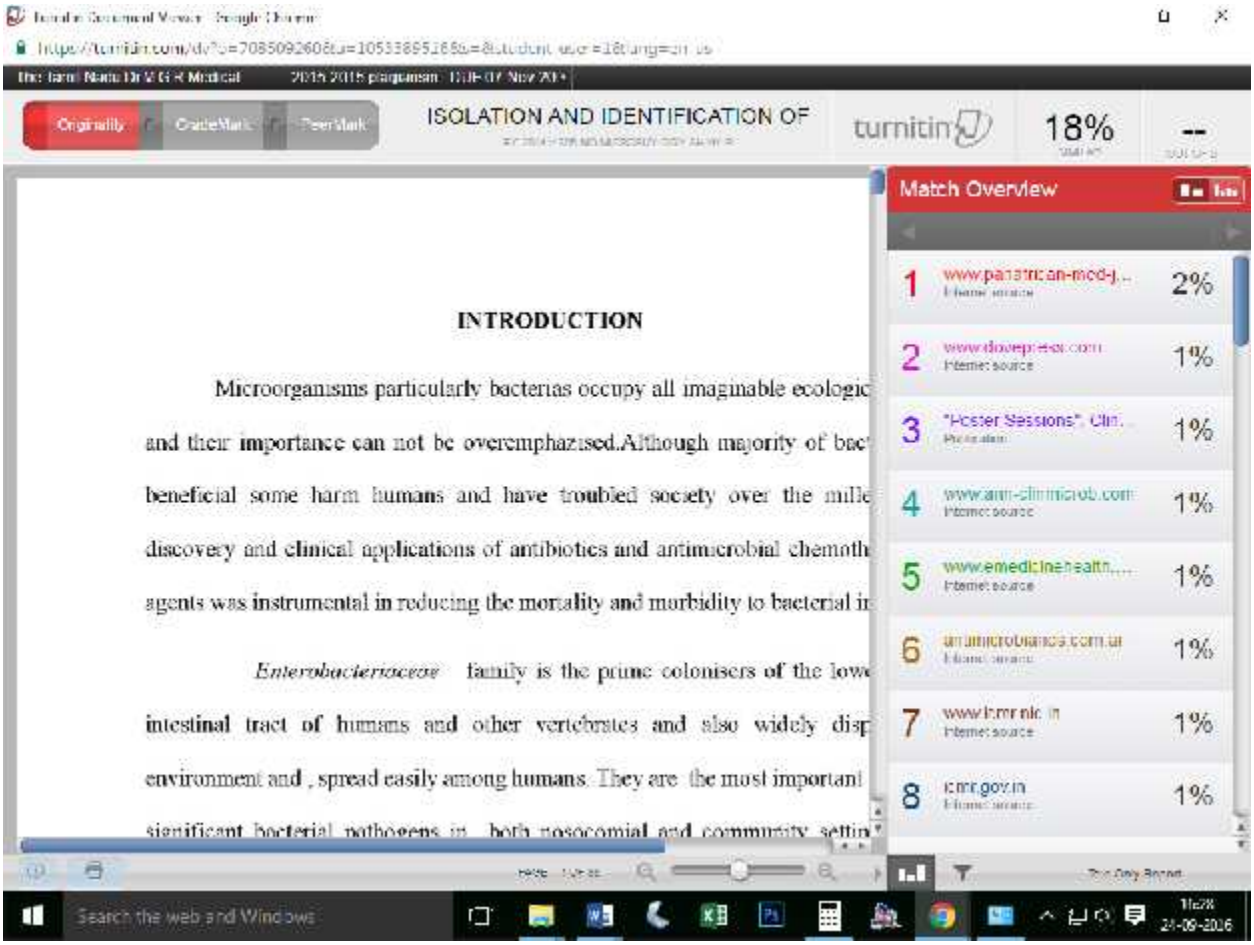
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INTRODUCTION
Microorganisms particularly bacterias occupy all imaginable ecologic
and their importance can not be overemphazised.Although majority of bac
beneficial some harm humans and have troubled society over the mille
discovery and clinical applications of antibiotics and antimicrobial chemoth
agents was instrumental in reducing the mortality and morbidity to bacterial in
Enterobacteriaceae family is the prime colonisers of the lowe
intestinal tract of humans and other vertebrates and also widely disp
environment and , spread easily among humans. They are the most important
significant bacterial pathogens in both nosocomial and community settin
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LIST OF ABBREVATIONS

E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
K. oxytoca	Klebsiella *oxytoca*
P. vulgaris	*Proteus vulgaris*
P. mirabilis	Proteus *mirabilis*
CRE	Carbapenem resistant *Enterobacteriaceae*
CSE	Carbapenem sensitive *Enterobacteriaceae*
MBL	Metallo beta lactamase
NDM	New Delhi metallo beta lactamase
CLSI	Clinical Laboratory Standards Institute
CDT	Combined Disk Synergy Test
MHT	Modified Hodge Test
EDTA	Ethylene Diamine Tetra Acetic Acid
E test	Epsilometer test
MIC	Minimum Inhibitory Concentration
ERT	Ertapenem
MP	Meropenem

MPI	Meropenem with EDTA
CFU	Colony Forming Unit
MHA	Muller Hinton Agar
ATCC	American Type Culture Collection
ESBL	Extended Spectrum Beta Lactamases
MDR	Multi Drug Resistant
Bla	Betalactamase
CSF	Cerebrospinal Fluid
Mg	microgram
PCR	Polymerase Chain Reaction
DNA	Deoxy Ribonucleic Acid
IC	Internal Control
CT	Cross Threshold

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INTRODUCTION

Microorganisms particularly bacterias occupy all imaginable ecological niches and their importance cannot be overemphazised. Although majority of bacterias are beneficial, some harm humans and have troubled society over the millennia. The discovery and clinical applications of antibiotics and antimicrobial chemotherapeutic agents was instrumental in reducing the mortality and morbidity due to bacterial infections. But the emergence and dissemination of superbugs has always been a global threat for control and treatment of infectious disease. The latest described superbug was NDM (New Delhi Metallo beta Lactamases), confer resistance to Penicillinins, Cephalosporins and Carbapenems.

Enterobacteriaceae family are the prime colonisers of the lower Gastro intestinal tract of humans and other vertebrates and also widely dispersed in environment and, spread easily among humans. They are the most important clinically significant bacterial pathogens in both nosocomial and community settings. They account for nearly 70% of urinary tract infections and 50% of septicaemia cases and are also responsible for a significant percentage of Alimentary tract infections[1]. These infections may be sporadic or occur as outbreak.

Members of the family *Enterobacteriaceae* are gram-negative, non

acid fast, non–spore forming, facultative anaerobes .These organisms are able to convert nitrate to nitrite by reduction, and able to generate catalase, but not oxidase, and are motile by peritrichate flagella or non-motile. They account for 80% of clinically momentous isolates of Gram negative bacteria and 50% of clinically significant bacteria

in microbiological laboratories, majority of them are urinary isolates[1]. These infections affect normal population as well as people with pre-existing illness.

Antibiotic treatment, using beta lactams is the stronghold therapy for Infections due to pathogenic *Enterobacteriaceae.* Antibiotic resistance among *Enterobacteriaceae* isolates, is a major health problem worldwide. Among the different types of drug resistance, multidrug-resistant (MDR) Gram negative bacteria is responsible for the greatest risk to human health because the promptness of development of drug résistance is much faster in Gram negative bacteria than of Gram positive bacteria as well as presence of only few drugs to treat these resistant bacterial infections.

Constant exposure of the bacterial pathogens to multiple antimicrobial agents has induced mutations that has lead on to the development of various drug resistant mechanisms for their survival .These mutant genes encoding enzymes capable of hydrolysing beta lactam antimicrobial agents in these bacteria, show poor activity against penicillins and cephalosporin antibiotics thus increasing the spectrum of difficulty to treat multidrug resistant bacterial infections.

Carbapenems are a family of beta-lactam antibiotics that exert their bactericidal action by inhibiting bacterial cell wall synthesis. These antibiotics exhibit a broad spectrum of activity and stability against various β lactamases produced by gram negative bacterias, including ESBL and AmpC β lactamases.

Carbapenemases are enzymes, having an ability to hydrolyse and inactivate a wide range of different antibiotics[2,3].The increased use and misuse of antibiotics in human medicine, agriculture and veterinary ,is the primary contributing factor for the development of this high level resistance. Other key risk factors for acquiring these

Carbapenemase resistant *Enterobacteriacea* are admission in transplantation units and intensive care unit as well as malnutrition, chronic debilitating illness, mechanical ventilation, prolonged hospitalization and prior surgical interventions [4].

In gram-negative organisms , two different types of Carbapenemase enzymes are accountable for resistance to carbapenem antibiotics, one that uses serine as the active site amino acid for hydrolysing the carbapenem *i.e.*, serine carbapenemases and the another type of carbapenemases that uses zinc ion at its active site i.e. Metallo-beta Lactamases or MBL[5] .Metallo-Beta Lactamase activity is inhibited by EDTA ,chelator of Zinc.

Most prevalent Carbapenemases in *Enterobacteriaceae* are KPC,VIM NDM, and OXA-48[6] and among this New Delhi metallo beta lactamase, an acquired, newly described superbug , confering resistance to broad range of antibiotics including carbapenams which are used as last resort for the treatment of Multi Drug Resistant *Enterobacteriaceae*[7,8,9.]

These resistance genes are carried either on chromosomes or associated with mobile DNA elements , carrying the *bla*NDM-1 gene, also have upto 14 other genes that confer resistance to most of the antibiotics including aminoglycosides, macrolides, and sulphamethoxazole, thus making these isolates multidrug resistant or, pandrug resistant. These mobile genetic elements also determine their spread by horizontal transference in clinically relevant gram negative organisms and produce resistant phenotypes[10] Antibiotics available for treating infections with carbapenemase – producing gram negative bacteria are the polymyxins (including colistin), tigecyclin and fosfomycin.

Castanheira et al. reported in 2011 that first case of bla NDM was identified in *Escherichia coli* , which was isolated in New Delhi, capital of India in 2006 ,While these pandrug resistant organisms disseminate to 50 countries covering all continents except South America and Antarctica, the horizontal transferability of the blaNDM-1gene via plasmids may be responsible for the rapid dissemination of NDM-1producing pathogens. In Chennai the prevalence rate of blaNDM-1 gene harbouring *Enterobacteriaceae* was 30 %, in Haryana it was 13 % and in UK it was 44%.[38] Carbapenemase producing *Enterobactericeae* strains are responsible for increased morbidity and mortality due to paucity of other treatment options which also enhance the development of further resistance.[11,12]

Timely detection and reporting to the clinicians are important for appropriate management of patients, development of antimicrobial surveillance programmes as well as for development of newer drugs, can prevent the transmission of this multi drug resistance.

Modified Hodge test,Combined disk assay,MBL E-test and Carbepenamase inactivation test are devised as phenotypic methods for carbapenamase detection. These assays shows low sensitivity and specificity for NDM[13]. False positive results may occur due to production of CTX-M with reduced outer membrane permeability. Metallo-beta lactamases are phenotypically detected by synergy with EDTA, these produce false positive results, therefore conformation by molecular methods is necessary.

Hence the present study is intended to detect the presence of MBL producing *Enterobacteriaceae* and the susceptibility pattern of these strains to other antibiotics in

clinical isolates in our hospital using both phenotypic and genotypic tests. All the *Enterobacteriaceae* isolates are subjected to four different phenotypic methods such as combined disc assay with Imipenem and EDTA, Modified Hodge test, MBL E test and carbapenemase inactivation test to find out their effectiveness in the detection of the production of MBL. The PCR is also performed to detect the presence of bla_{NDM-1} gene which is responsible for production of New Delhi Metallo beta lactamases.

2. AIMS AND OBJUCTIVES

- To systematically screen the clinical isolates of multidrug resistant *Enterobacteriaceae for* resistance to Ertapenam.

- To determine MIC by E-test with Ertapenam strip.

- To detect enzymatic activity of Metallo beta lactamase phenotypically by modified Hodge test ,Combined disc test, MBL E-test and carbapenemase inactivation test.

- To study the prevalence of blaNDM-1 among carbapenemase producing *Enterobacteriaceae* .

3.REVIEW OF LITERATURE:

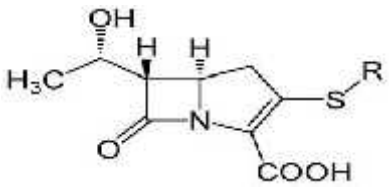
3.1 Introduction

Carbapenem antibiotics are decisive drugs, which are considered to be the last resort antibiotics for the treatment of multi drug resistant bacteria infections[37]. The end result of increased use as well as misuse of carbepenems is the reason for the development of first carbapenem resistant *Enterobacteriaceae* (CRE) in 1993. [33] New Delhi metallo beta lactamase-1 producing superbugs are the emerging multidrug resistant or pandrug resistant gram negative bacterial pathogens capable of hydrolysing and inactivating carbapenems.[14] The infections due to these pandrug resistant organisms pose a complex task in treatment because they have only limited therapeutic options[15]. So rapid and reliable detection methods are mantatory in patients infected or colonized with carbepenemase releasing strains ,which also have essential role in infection control as well as outbreak investigation.

3.2. Carbapenems:

Carbapenems are beta-lactam antimicrobial agents with a broad spectrum of activity because of the hydroxyethyl side chain present in it.[6] They exibit their bactericidal effect by inhibiting the synthesis of peptidoglycan,[25]. The carbapenems were developed to overcome antibiotic resistance mediated by earlier bacterial beta-lactamase enzymes like ESBL and Amp C beta lactamases.

3.2.1 Structure of Carbapenem antibiotics:



3.2.2. Mechanism of action

Carbapenems not sucessfully diffuse through the cell membrane, but these antibiotics enters the periplasmic space through porins **,**bind to PBP1 and PBP2 and permanently acylate the PBP ,then inhibit peptide cross linking as well as other peptidase reacions and leads to lysis of the bacterial cell[5].They are stable against most plasmid-mediated or chromosomally mediated β lactamases except those produced by *Stenotrophomonas maltophilia* and some strains of *Bactericides fragilis*.[6] The structure of an acyl−enzyme intermediate for Imipenem helps the antibiotic to fight against β-lactamases.[8]

3.2.3. Spectrum of activity of Carbapenams:

Carbapenems have a wide spectrum of activity against Gram-positive, Gram-negative and anaerobic bacteria and are often used as end line agents.Imipenam,Panipenam and Doripenam are most effective antibiotics against Gram positive bacteria and Meropenam,Biapenam,Ertapenam and Doripenam are slightly more active againt gram negative bacteria. More than 90% of Enterobacteriaceae, including those with reduced susceptibility to third generation Cephalosporins,Cephalosporins with beta lactam inhibitors Gentamicin and Amikacin,are susceptible to Carbapenems.

3.2.4. Clinical Indications

Imipenem

Imipenem was indicated in infections caused by hospital acquired , multidrug resistant organisms. Cilastatin, a peptidase inhibitor, should be given with imipenem,because this drug was hydrolyzed by dihydropeptidases in renal tubules.

Meropenem

Meropenem is superior to imipenem because of its reduced potential to cause seizures. The enzyme dipeptidase cannot destroy meropenem.It is also used in the management of hospital acquired, worrisome infections.

Ertapenem

Ertapenem can be given as a single dose a day because of its long half- life. The unique antimicrobial spectrum and pharmacokinetic properties of ertapenem make it more suited to treatment of patients with community acquired resistant organisms and on outpatient intravenous antimicrobial therapy. It has poor activity against non fermentors.[1]

Doripenem

Doripenem is active against Non fermenters because it harbour sulfamoylamimoethyl-pyrrolidinylthio group in its side chain. The affinities for penicillin binding proteins are species specific in doripenem. For example, doripenem has affinity for PBP3 in *Pseudomonas aeruginosa*.

Oral carbapenems like tebipenem pivoxil and faropenem are given as prodrugs to increase intestinal absorption and get activated by host enzymes in the liver or intestinal wall[63,103].

3.2.5. Mechanism of carbapenem resistance:

Resistance to carbapenems develops

- Due to structural changes within their PBPs.
- Due to production of beta lactamases such as metallo-beta-lactamases and serine containing Carbapenemases , or
- Due to decreased drug entry consequent to mutations altering the outer membrane porins[62]. Among these three mechanisms, carbapenemase production plays a most

important role .As it can be transmitted via plasmids rapid dissemination is possible to other sensitive bacteria and poses a threat to treatment strategies.[60,61]

3.3 New Delhi metallo-beta-lactamase-NDM :

The New Delhi metallo-beta-lactamase (NDM) is a challenging, new metallo beta lactamase isolated worldwide.[17] .The gene NDM encodes a 269 amino acids containing protein, with a molecular weight about 27.5 kDa[16]. Till date 13 types of NDM have been identified (NDM-1 to -14).NDM-11 was not assigned to any unique variant[26-28]. The differences arise as a result of point mutations within the NDM gene [18-24]. The site of mutation on the gene predicts the rate of hydrolysis of carbapenams. As the mutations are not located in the active site of the enzyme, NDM-2 and NDM-3, have similar hydrolytic activity like NDM-1 . Whereas NDM-4 has mutations in the region coding for the active site they cause increased hydrolysis of carbapenems.

3.4 The emergence of NDM-History:

The New Delhi metallo-beta-lactamase (NDM-1) was first described by Yong et al. in December 2009 ,and was named after the City of origin NewDelhi[34].The NDM-1 enzyme was was first isolated from a Swedish patient of Indian origin who was treated in New Delhi for gluteal abscess.Subsequently he developed a multidrug resistant UTI. On returning back to Sweden he was hospitalised in Orebro, Sweden, in January 2008 ,his urine culture yielded an isolate of carbapenem resistant *Klebsiella pneumonia.* Subsequent stool samples from the same patient, tested in March 2008 yielded a carbapenem resistant strain of Escherichia coli[17] due to genetic transfer of drug resistance. Phenotypic testing of both isolates showed it was due to a metallo-b-lactamase (MBL) but PCR analysis failed to detect known MBL genes. Further Cloning and sequencing studies identify that the resistance was due to a new metallo beta

lactamase enzyme which shared only 32% identity with VIM-1/2. The novel MBL was designated NDM-1, as the authors of the report believed the resistance originated from India[25].

In March 2010 ,a hospital in Mumbai isolated the newer, blaNDM-1 gene from most of the Carbapenem non susceptible bacterias .[6] In June 2010, the Centers for Disease Control and Prevention (CDC) reported its first three cases of NDM-1 producing *Enterobacteriaceae* isolates from patients who had received recent medical care in India[35] . Three cases of *Acinetobacter baumannii* harbouring bla NDM-1 were isolated from an intensive care unit of a hospital in Chennai, in April 2010. First confirmed case of NDM superbug August 21, 2010, Ontario, Canada had the "superbug" in Brampton. They also found other confirmed cases in British Columbia and Alberta[36].

A study report in August 2010 issue of the journal The Lancet Infectious Diseases showed the emergence and spread of bacteria carrying the bla NDM -1gene. This reported about 37 cases in the United Kingdom, 44 isolates with NDM-1 in Chennai, 26 in Haryana, and 73 in various other sites in Pakistan and India[38].

According to **Nordman** *et.al* bacterias harbouring NDM-1 are not only reported from India but also in more than 50 different countries [39, 40]. But many of the patients carrying NDM-1 have a history of medical tourism to India, Pakistan or Bangladesh. NDM-producing bacterial infection patients having only intercontinental travel without any association with health care delivery system indicates the possibility of cross infection .

Studies conducted by **Kumarasamy *et,al*, Walsh** *et,al* &**Yong** *et,al* showed the epidemiological association, between NDM – 1 positive strains and Indian subcontinent [(10, 43,44)]. **Kumarasamy KK** *et al* in India reported that the majority of NDM-1-producing

Enterobacteriaceae were due to local cross infection [10, 45].

On January 12th 2011, the editor of The Lancet, apologized and acknowledged for naming a superbug after New Delhi was an "error"[46,47,48]**Dortet** *et al* in 2012 highlighted that the Middle East Asia is a secondary reservoir for NDM-1 positive isolates in which, IncX3 plasmids may play a significant role in transmission[58].Several reports from incongruent parts of the world confirm the possibility of local acquition of organisms harbouring blaNDM .[49-57]

Poirel *et, al* reported that presence of IS*Aba125* element inbetween *bla* $_{MBL}$ gene responsible for biodiversity and associated global pandemia.

Moellering RC *et.al* found the presence of associated genes with bla NDM -1 gene confer fight againt antibiotics, like erythromycin, ciprofloxacillin, rifampicin and chloramphenicol. It is also accompanied by a gene responsible for the production of broad spectrum beta lactamase (CMY -4). [29]

Kumarasamy *et al*, found found the relationship between blaNDM -1 and other genes such as blaOXA- 23 and armA .[10].A similar study by **Poirel** *et al*, observed that the NDM - 1 gene in a strain of *Citrobacter freundii* was accompanied by 9 different types of beta lactamase[32].

Yong *et,al* and Deshpande *et,al* highlighted that gene encoding biaNDM-1 is a plasmid mediated genetic element and capable of more complex and unpredictable spread than the gene encoding KPC [17,33].

3.5 Factors responsible for production of New Delhi metallo betalactamases:

The genetic code (bla_{NDM-1}) located on either a plasmid or integrated into the bacterial chromosome is responsible for the synthesis of the enzyme NDM-1. Researchers suggest that

environmental pressures, such as the use or overuse of antibiotics, selected for bacteria that could synthesize this enzyme to survive. Some speculate that because un restricted use of antibiotics in many countries, responsible for the development of antibiotic-resistant strains with NDM-1, some reports suggest India is where this genetic element first developed.

3.6 Methods of transfer of gene encoding NDM:

Tranfer of genes among bacteria is crucial in generating diversity,allowing bacteria to adopt quickly to changing environments.Mobile genetic elements such as plasmids and transposons are important agents for transfer of antibiotic resistantance determinants beween bacteria .Bacteria can transfer genetic information to get protection against most antibiotics through mutation or via horizontal transfer by Transformation Transduction or Conjugation. The most common of these methods of transferring antibiotic resistance is conjugation.

3.7 Mode of transmission:

Yong et, al reported that NDM-1- positive bacteria are capable of colonising the Gastro-intestinal tract of humans for prolonged periods and are spread through contamination of water and environmental surfaces.[17]

The more frequent international travel has contributed additionally to rapid global dissemination. **Kumarasamy** *et al* and **Borgia** *et,al* reported that recent hospitalisation in, Indian subcontinent is identified as a risk factor for colonisation and infection with the NDM-1 producing strain.[10,95]

The infection is also spread by contaminated medical equipment and by physical contact between patients and healthcare personnel due to the development of resistance against standard sterilization procedures used on medical equipment. Medical procedures, such as the prostate biopsy can also play a role in transmission.

Leverstein *et,al*. in his study in 2010 reported travel-associated cases but without any contact with healthcare systems also acquired NDM-1 in the community[92,93] **Kumarasamy** et,al .in 2010 described that inter-human transmission is likely to play a major role, via contaminated hands, food or water, particularly in resource poor countries In India,the majority of NDM-1-producing *Enterobacteriaceae* were community-acquired[10, 94] .

Nordmann *et,al* reported that *Enterobacteriaceae* family members have been identified as important nosocomial pathogens; infection can lead to severe morbidity and mortality, particularly in intensive care units (ICU),internal medicine and surgical units, and pediatric units[63] **Kumarasamy** *et,al* **Nordmann** *et,al* & **Poirel** *et,al* reported that NDM producing organisms responsible for both hospital- and community-acquired infections[(10,63,66)].

Kumarasamy *et,al* **Nordmann** *et,al* and **Wilson** ME,*et al* described that NDM-1 producing bacteria are responsible for urinary tract infections, pneumonia, septicaemia, wound infections and device-associated infections[(10,65.66)] .

Bacteria from the *Enterobacteriaceae* family are the most common cause of urinary infections. They can also cause bloodstream infections (sepsis), pneumonia, or wound infections. Clinical manifestations reflect the site of the infection[1]. Most patients will have fever and fatigue. If bacteria enter the bloodstream, patients may have toxic symptoms . Symptoms are similar to bacteria that express NDM-1 and those that do not express NDM-1. So patients who harbour NDM-1 will not respond to most conventional antibiotics so they are at high risk for complications.

Shenoy *et al* reported that NDM positive MDRGNB responsible for 33.33% of the total

Respiratory tract infections and 60% of the total Urinary tract infections[67].

NDM -1 positive organism can get colonized in the gut in the absence of any feature of disease. As most of these organisms are normal commensals of the gut, the screening of these organisms is a difficult job[24].

3.8 RISK FACTORS:

Important risk factors include, prolonged hospital stay,ICU admission, surgical procedures or the use of medical devices like ventilators or catheters are implicated in the spread of drug-resistant superbugs also associated with situations such as being transferred between hospitals as well as presence of diabetes mellitus [15,16].

Studies from **Yong d**.*et.al*, **Mulvey MR** *et al*, **Zarfel G** *et,al*. **Koh TH** *et,al* ,**Gottig S** *et.al*, **Hammerum** et,al ,**Leverstein** et.al,and **Kumarasamy** et .al shows that most of the NDM-1 cases were associated with co-morbidities, advanced medical procedures like organ transplantation, bone marrow transplantation and cancer chemotherapy and invasive surgical procedures[10, 17, 96-102]

. **Anjana** et al. described that the overcrowding of a number of critically ill patients within a relatively small enclosed area and use of sophisticated invasive machinery such as ventilators and catheters increase the proportion of patients who are unusually susceptible to infection and who become reservoirs for their spread[67].

3.9 PREVALANCE:

3.9.1 **GLOBAL PREVALENCE:**

In 2010 Deshpande et, al reported the increasing prevalence of Carbapenemase producing *Enterobacteriaceae* from 0% to 8% between 2006 to Aug 2009 in ICU blood cultures.

In 2013 J. **Kamile Rasheed** et,al from CDC reported 8 bacterial strains having NDM-1, and one isolate with *bla*NDM-6 gene.

Toleman et, al. in 2015 in Bangladesh, found the prevalence among $bla_{\text{NDM-1}}$ carrying GNB was 62% in 2012.

Yamaga et,al in 2015 reported, the prevalence of 47.8% for KPC, 8.7% for VIM while 21.7% of bacterial strains have co-existence of the NDM-1 and VIM genes in Nigeria**.**

Fifty-two carbapenem non-susceptible isolates were screened with NDM-1 specific primers .Of these,23% were positive for *bla*NDM-1 . The 12 isolates positive for *bla* NDM PCR were: six *Klebsiella pneumoniae*, four *Escherichia coli* and two *Enterobacter cloacae*. Full gene sequencing also confirmed that the genes encoded NDM-1 **Teo** *et al.*

Moi *et .al* reported that commonest CRE was NDM-1 seen in 44.4 % of *Enterobacteriaceae* followed by KPC accounts for 39.9 % of isolates while OXA-48 responsible for 7.8 % in Singapore.

Zainol *et al* reported 29% of NDM-1 producing Enterobacteriaceae in Malaysia

N. Stoesser *et.al* reported in 2014 that prevalence of *bla*NDM was 7% among *k.pneumoneae* strains in Nepal.

Sahin *et al.* ,reported in 2015 that the prevalence of NDM-1 was 2.3% in Turkey

3.9.2 INDIAN SCENARIO

Various studies from India quoted the prevalence of MBL varying from 8-14% (**Bashir** *et al*[8]., 2011; **Varaiya** *et al*[58]., 2008; **Hirakata** *et al*[59]., 1998).

Ram Gopalakrishnan *et al* in his study on a retrospective review of surveillance over the period 2001-2008 reported the antimicrobial resistance patterns in Indian hospitals have a high prevalence that up to 40% of *Pseudomonas* was resistant to carbapenems.

Vikas kumar *et al* revealed in his study from North India that 87.17% of resistant isolates were metallo beta -lactamase producers [64]. Noyal et al from Pondicherry reported 50% of the resistant isolates were MBL producers[65].

Study done by **walsh** *et,al.* in 2011 in New Delhi showed the prevalence of NDM-1-producing *Shigella boydii* and *Vibrio cholera* in 4% drinking water samples and 30% of seepage samples signifying the possibility of cross infection .

Study by **Castenheira** *et al* showed that prevalence of *bla*NDM-1 was 38.5% among CRE in India isolated between 2006-2007.

Mohan *et al* in 2013 reported the prevalence that among the isolates obtained during2012,*bla*NDM-1 gene was present in 53.4% of total isolates and 84.3% of *Enterobacteriaceae*[8]

Walsh *et, al* in 2010 reported that prevalence data in India for NDM-1 is 2–8% in his study.

Seema *et al*.,2011 reported that study undertaken in 2010 at a tertiary referral hospital in Varanasi in north India, 6.9 % of clinical isolates of Enterobacteriaceae (comprising 30 Escherichia coli, 12 *K. pneumoniae* and 12 *Citrobacter species*) were positive for the blaNDM-1 gene.

Bashir *et, al* in his study reported that 60% of the metallo beta-lactamase carrying organisms were found to be NDM-1 producers in 2014 in Srinagar, Jammu & Kashmir.

Indian study by **Karthikeyan** *et, al* showed a percentage NDM-1 was 30 per cent in Chennai, 13 per cent in Haryana and 44 per cent in US among *Enterobacteriaceae* [38].

Shenoy *et al* in 2014 in his study reported the prevalence of NDM-1 positive *Enterobacteriaceae* was 48.57%.

3.9.3 Prevalance **in Tamilnadu:**

Ramesh et, al in 2014 reported that prevalence of NDM –I among GNB was 12.8% in Tamil Nadu

Shanthi et, al reported in 2014 that prevalence of NDM-1 gene is 6.55% in Pseudomonas in Tamil Nadu.

3.10 DETECTION METHODS:

Kumarasamy KK *et.al*, **Yong** *et, al*. & **Deshpande** *et, al* described in 2010 that, Laboratory detection methods of NDM -1 involves, isolation and identification of bacterial strains by gram staining, biochemical characteristics and cultural characteristics then , conventional susceptibility testing followed by enzyme assays and molecular detection of

bla NDM -1. [10, 17, 18,103]

3.10.1 Phenotypic methods:

Phenotypic methods are cost effective, easy to perform, interpret, and introduce into the work flow of a clinical laboratory. But these methods are not ideal because they can be affected by growth medium, phase of reproduction and rate of spontaneous mutations.Therefore, genotyping techniques are preferentially used worldwide.

3.10.1.1 Kirby Bauer disc diffusion test:

Doyel *et, al* reported the resistance to ertapenem, sensitivity 97.7% and specificity 100%, for MEM sensitivity 93% and specificity 90.7 % and IMP sensitivity79.1 % and specificity 88.4 % resistance[104]

Nordmann *et al* recommended that Ertapenem is a most appropriate carbapenem for detecting NDM-1 producers[18].

3.10.1.2 Modified hodge test:

The modified Hodge test was a suggested screening assay by the Clinical and Laboratory Standards Institute (CLSI) for carbapenemase releasing organisms[106].

According to CLSI document this test is relatively cheap, rapid and easy to perform and has been reported to have a detection sensitivity exceeding 90% for KPC-type carbapenemases and 11% for metallo-β-lactamases.[105]

Edelstein M, et al reported in 2010 that, MHT only a phenotypic screening test for detection of hydrolysing activity of Carbapenems , and therefore will not differentiate various types of carbapenemases. By this method detection of MBL can be difficult that pose unique challenges in the laboratory[31]

Doyle D *et al* in 2012 found that the modified Hodge test had a sensitivity of 98% for finding carbapenemases particularly KPC, but it was not good for finding metallo- β-lactamases. The specificity of the test was 93%.[33]

Thompson *et, al* in 2010 reported it is a less sensitive and time consuming assay for detection of MBL activity. [112]

Rimrang B *et al* reported in 2012 that more than 50% of the NDM-1 positive *Enterobacteriaceae* strains were MHT-positive than KPC-producing strains[111]

Andrea Bartolini *et,al* reported in 2014 that MHT for carbapenemase **producers** shows 94% sensitivity and 100% specificity. KPC-producers shows 100% sensitivity, 100% specificity but the NDM-1 positive isolates used to show a distorted inhibition zone .[110]

Mochon AB et *al* reported in 2011 that NDM-1 producers used to give

less strongly positive MHT results.[109]

Diana Doyle *et al* reported that the specificity and , sensitivity of carbapenemase-negative isolates were 93% and 12 %. The sensitivity for KPC producers was 98% and 93% for OXA-48-like enzyme producers but the **sensitivity is only for** NDM **producers was 12%**[104]

Arif Sultan *et.al* reported in 2013 that sensitivity of Modified Hodge Test was 42.8%, and specificity was 69.8% with a positive predictive value of 94.2% and a negative predictive value of 9.6%. [108]

Nor Zanariah *et, al* reported that the sensitivity and specificity of the MHT for NDM-1 producers among *Enterobacteriaceae was* 100% and 40%, respectively.[107]

Tzouvelekis *et al* reported that sensitivity for modified Hodge test was 61% and specificity was 93%.[106]

3.10.1.3 Combined **disc test:**

In combined disk assay inhibition zone around the carbapenem disk, is increased in the presence of chelators like EDTA. This test shows most sensitivity against metallo beta lactamse production.[115]

A study conducted by **Savitha** *et, al* in 2014 in Karnataka reported that the sensitivity and specificity for EDTA based phenotypic method for MBL detection is 57.9% and 78.94%.[113]

Doyle *et,al* reported that sensitivity of combination disk assay was 78% and specificity was 93%.[104]

3.10.1.4 **MBL E-Test:**

Yan JJ *et.al* in his study highlighted incubation of bacterial strain with Etest strip impregnated with imipenem and imipenem-EDTA combination showing three or more two fold dilution in the presence of EDTA indicates the production of metallo-beta-lactamase, Chance of false negative result was more if the MIC of imipenem is less than 4 micrograms/ml [115].

Franklin *et.al* described that Etest MBL was reliable for screening of the hydrolysing activity of carbapenemase enzyme.[31]

Doyle *et, al* reported that sensitivity of MBL E-test was 63% for NDM-producing *Enterobacteriaceae* but specificity was 100%[104]

Nordmann *et, al.* described MBL was successfully detected by the EDTA inhibition test, using E -test strips impregnated with imipenem and imipenem with EDTA. [114]

Kaleem F, *et al* in his study reported that the MBL E test can be used as a confirmatory test for MBL detection[117].

A study by van der Bij *et al* reported that by comparing the CDT with the E test for the MBL production the sensitivity and the specificity for the E test was 100% and 95% respectively[118].

3.10.2 Genotypic methods:

Nordmann et, al in 2009 described that phenotypic assays failed to detect isolates carrying multiple carbapenem resistant genes, which were successfully characterise only by genotypic analysis. Phenotypic methods are growth dependent and time- consuming as it takes 16 – 24 hours to completion, not useful in clinical aspects and interpretation of results are also subjective[6]. Therefore, identification and by molecular methods such as real

time-PCR have been shown to be sensitive and more accurate for identification of carbapenemase genes.

Doyle *et.al* the reported prevalence likely under represents the problem, because of the decreased sensitivity of the phenotypic methods molecular methods are more sturdy also found that PCR has a sensitivity and specificity of 100% for NDM detection. [119]

Doyle D *et al* in 2012 evaluated the performance of the in-house multiplex PCR is highly sensitive and specific for the recognition of blaKPC, blaNDM, blaOXA-48-like, blaVIM, and blaIMP [119]**.**

Kaesse M *et al* in 2012 found that the sensitivity of polymerase chain reaction was 100% for blaKPC, blaVIM, blaNDM, and blaOXA-48.[124]

3.11 TREATMENT:

Kumarasamy *et,al* highlighted that Colistin have good in vitro activity against NDM-producing isolates ,have 89%–100% susceptibility and for Tigecycline susceptibility is about 56-67% [10]

Tigecycline posses good outcome in 70% of the patients infected with carbapenemase producers, the increase in mortality among patients using tigecycline than other biocides .[120]

Susceptibility of Tigecycline against Nonfermenting Gram-negative organisms was low about 15%, as compared to 60% for *Enterobacteriaceae*, as reported by **Jain** *et al.*[41]

Jamal et *al* reported in 2013 that colistin is nephrotoxic, and tigecycline, a bacteriostatic drug, so not suitable in sepsis. In addition, tigecycline is not suitable for treating urinary tract infections because of ineffective excretion via urine [125].

Study conducted by **Mac Vane** SH *et al* in 2014 compared Ceftazidime and Ceftazidime with Avibactam against NDM producing and other beta-lactamase producing isolates.But they showed negative result against NDM-producing isolates.[121]

Susceptibility of NDM-releasing strains to fosfomycin was 60.5[123] to 78%.[124].

Zmarlicka MT *et, al* in his study he confirmed the need of high dose of doripenem as infusion that produce better response compared to a standard dose of 500 mg fre thrice daily.

combination therapy using carbapenems and other compounds shows good response against NDM producers also suggested that combination therapy should continue to be included in the treatment strategy against infections due to NDM producers.[122]

Cobo *et, al.* described that a combination of tigecycline and colistin synergistic action in multi drug resistant isolates [126]

Livermore DM et, al reported in 2010 that combinations using monobactums such as aztreonam which have a ability to resist hydrolysis by metallo beta lactamases are recommended by some studies. NDM - 1 containing bacterial strain may also have other carbapenemase like amp C or ESBLs which may hydrolyze monobactams. So inhibitors of these enzymes (like NXL104) should be used along with combination therapy [127].

Falagas ME et, al. in 2010 reported that Fosfomycin is also suggested for use in Carbapenemase producing pan resistant Enterobacteriaceae [128].

Tangden *et al* described that combination of fosfomycin and colistin was found to have both bactericidal and synergistic activities after 24 hours in a strins resistant to fosfomycin. Similar activity was seen with a Combination of Rifampin, Meropenem, and Colistin eventhough both strains are resistant to Rifampin or Meropenem alone.[129]

3.12 **PROGNOSIS:**

Factors determining outcome :

Kumarasamy *et; al* highlighted in his study that although a drift towards positive outcome is more, there are many other host related factors that contribute to the patient's outcome,includes

- immune status of the patient
- underlying co -morbid illness
- appropriate treatment, If the NDM-1-producing bacteria are susceptible to colistin, the prognosis is usually good
- elimination of underlying problems such as drain or remove abscesses and. infected necrotic tissues
- the management of invasive devices that might be colonized with the pathogen.
- the patient on respirators, immunocompromised patients, and patient's in Intensive-care units have a more guarded prognosis.[10]

N. Stoesser *et, al* in 2014 reported that the outbreak-associated mortality was about 64% of inpatient deaths in 25 neonates, in contrast with a hospital-wide contemporaneous neonatal death rate of 0.7%. The neonatal critical care mortality rates were 46% during the outbreak period.

In a study by **Bores A** *et al*, observed that crude mortality and attributable mortality in the patients with carbapenemase producing *Klebsiella pneumonia* bacteraemia was 71.9% and 50% respectively. [131]

In a similar study by **Patel G** *et al*, found that mortality among the cases with carbapenemase resistant *Klebsiella pneumoniae* was significantly more as compared to control (40% Vs 20%) .[132]

Munoz *et,al* reported that mortality rates for *K. pneumoniae* carbapenemase infections being between 42% and 53%,[20]the mortality rate seen with NDM seems staggeringly low.

4. MATERIALS AND METHODS

In the present study 195 Multidrug resistant *Enterobacteriaceae* isolates obtained from various clinical specimens like Pus, Urine, Blood, Sputum, Fluids, Swabs and vascular catheter tips from various departments in Tirunelveli Medical college, Tirunelveli were included in this study to establish the prevalence of blaNDM-1 among them.

4.1 Study area:

The present study was conducted at the Department of Microbiology, Tirunelveli Medical College.

4.2 Specimen :

All clinical samples were obtained from Microbiological Laboratory (Blood, Urine, Pus, Swabs, Stool, Sputum, fluids, catheter tips).

4.3 Sample size :

195 non duplicate clinical isolates of Multidrug Resistant *Enterobacteriaceae* isolates in the above specimen.

4.4 Study population:

Samples obtained in the Department of Microbiology in Tirunelveli Medical College.

4.5 Study period :

8 months from December 2015 to August 2016

4.6 Study design : Prospective study

4.7 Ethical committee approval:

This study was carried out after approval by the Institutional Scientific and Ethics Committee.

4.8 **Informed consent:**

Informed consent was obtained from all persons involved in the study.

4.9 Proforma**:**

A filled in proforma was collected from the patients with details like name, age, sex, ward, clinical diagnosis, risk factors, surgical interventions, hospital stay and other parameters relevant to the study.

4.10 Methodology:

4.10.1Sample collection and processing:

A total of 2514 samples received at Microbiological laboratory were screened during the study period. Of which 425 isolates were *Enterobacteriaceae*. Out of this, 195 isolates were multidrug resistant isolates, resistant to more than three groups of antibiotics detected by Kirby-Bauer disc diffusion technique with beta lactam and non-beta lactam antibiotic containing discs. Drug susceptibility was interpreted according to CLSI guidelines. Carbapenem resistance was detected by Kirby Bauer disc diffusion test using Ertapenem disc and E-test. Those isolates were subjected to metallo beta lactamase detection methods such as Modified Hodge test, Combined disc test(CDT), MBL E-test, and Carbapenaemase inactivation test and detection of blaNDM-1 was done by Multiplex PCR.

4.10.2 Sample storage

MDR *Enterobacteriacea isolates* were sub-cultured on to nutrient agar slope and stored at 2 to 8˚C. The isolates were sub-cultured every fortnight.

4.10.3. Safety precautions

All the procedures were carried out in a Biosafety cabinet with due precautions.

4.10.4 **Antibiotic susceptibility testing**:

According to the CLSI guidelines the antibiotic susceptibility testing was done in the entire *Enterobacteriaceae* isolates by Kirby Bauer disc diffusion method.

4**.10.4.1. Inoculum preparation**

Inoculum was prepared by direct colony suspension method by taking four to five well isolated colonies of *Enterobacteriaceae from* 18-24 hours culture, in peptone water to achieve a turbid suspension.

4.10.4.2. Inoculum standardisation

Using Wickerham's chart the turbidity of the inoculum was adjusted to 0.5 McFarland standard ($1.5x10^8$ CFU/ ml).

4.10.4.3. Kirby-Bauer's disc diffusion method:

1. Prepare a bacterial inoculum of 0.5 Mc Farland standard
2. A sterile cotton swab was soaked in the inoculum and a lawn culture was made on to the Muller-Hinton agar (MHA).
3. The panel of antibiotic discs were dispensed (Ceftriaxone disk 30 µg, Cefotaxime disk 30µg, Ceftazidime disk 30µg, Ceftazidime with clavulanic acid(30/10)disk, Gentamicin disk 10µg, Amikacin disk 30 µg ,Ciprofloxacin disk 5µg, and Cotrimoxazole disk (1.25/23.75)

4. Zone of inhibition was recorded and interpreted as per the CSLI guidelines.

4.11.5 Detection of carbapenemase resistance:

4.11.5.1 Disc diffusion method:

According to CLSI guidelines disc diffusion test for Ertapenem(30 µg) was done for all Multidrug resistant *Enterobateriaceae* isolates(195 isolates).Zone of inhibition was also determined according to that.

4.11.5.2 Ertapenam E-test:

It is a quantitative assay for determining MIC concentration of Ertapenem effective against that isolate.

Procedure:

Ertapenem E-strip was applied to the surface of the lawn culture prepared by the tested organism and incubated for 18-24 hrs at 35◦C .MIC is calculated at the point where the edge of the inhibition eclipse intersects the strip MIC Test strip.

4.11.6 Screening of Metallo beta lactamase production:

All Ertapenem resistant isolates were subjected to metallo beta lactamase detection methods such as Modified Hodge test, Combined disc test, MBL E-test and Carbepenamase inhibition test.

4.11.6.1 Modified hodge test:

MHT was performed according to the standard CLSI guidelness.

Procedure:

- A Muller Hinton agar plate is inoculated with 1:10 dilution of a 0.5 Mac Farland suspension of E.coli ATCC 25922 bacterial and was streaked for confluent growth using a swab .
- A 10µg Imipenem disk was placed in the centre of the M-H plate.
- The test isolate was streaked as a straight line from the disc to the edge of the plate.
- Plate was incubated at 35◦ ± 2 ◦ for 16-24 hrs.

Interpretation:

Presence of a distorted inhibition zone is interpreted as positive result for carbapenemase hydrolysis screening.

4.11.6.2 Combined disk test :

Phenotypic detection of MBLs was done by combined disk test.

Procedure:

- Two imipenem disks (10 µg), one containing 10 µl of 0.1M anhydrous EDTA (292 µg), were placed on a plate inoculated with the test organism.
- Inhibition zones of the Imipenem and Imipenem with EDTA were compared after 18-24hr of incubation at 37degree.
- A zone diameter difference ≥5 mm between Imipenem and IPM-EDTA disc was interpreted as positive results for MBL production[31].

4.11.6.3 MBL E Test:

MBL E test strip is used according to the recommendations of the manufacturer, where one sideof the strip is coated with mixture of Meropenem +

EDTA and Meropenem in a concentration gradient manner. The strip is made of porous material and the antibiotics are distributed evenly on either side of the strip. E test MBL strip has a double sided antibiotic concentration in a range of Meropenem (MP) 0.125 to 8 µg/ml and Meropenem + EDTA (MPI) 0.032 to 2 µg/ml with a fixed concentration of EDTA.

Procedure:

1. Prepared a bacterial suspension of 0.5 Mc Farland standard
2. Bacterial suspension was inoculated as a lawn on to the M-H agar .
3. The MBL E test strip container was taken from the freezer and kept at room temperature for 15 minutes before opening .
4. The strip was then taken with a sterile forceps or E test applicator and applied to the dried agar surface with the MIC scale facing upwards
5. The plate was incubated aerobically for 16-18 hrs at 37^0C.

Interpretation:

A reduction in the MIC of Meropenem of three or more 2-fold dilutions in the presence EDTA is interpreted as a positive test indicative of MBL production.

4.11.6.4 Carbpenamase inactivation test:

Procedure:

- Prepared a test bacterial suspension
- Place 10µg meropenem disc inside the bacterial suspension.
- Incubate for 2hrs at 35◦C
- Place that meropenem disc on M-H agar inoculated with E.coli ATCC 25922
- Incubate for atleast 6hrs at 35◦C.

- Read presence or absence of inhibition zone.

Interpretation:

Presence of inhibition zone: no MBL activity

Absence of inhibition zone: MBL activity

4.11.7 MBL detection by PCR:

The Ertapenem resistant Enterobacteriaceae *isolates* were further tested for *blaNDM* -1 gene by Real-Time PCR. The PCR kit was procured from Helini Biomolecules, Chennai, India. According to the manufacturer's instructions, the procedure was performed.

Table 1: Requirements

Equipment	Reagents& media	Supplies
- Thermocycler - Analysis software - Water bath - Ultracentrifuge - Vortex mixer	- DNA Extraction kit - Helini blaNDM-1 PCR kit - Nuclease free water	- Micropipettes Pippet tips - 1.5 ml microcentrifuge tubes - 96 well reaction plate - Spin column

Storage and stability:

- The bacterial genomic DNA extraction kit was stored at room temperature.
- The proteinase K and Lysozyme were stored at -20 ^{0} C.

4.11.7.1 Bacterial DNA Extraction:

About 4-6 colonies of the test strains were inoculated in peptone water and incubated at 37^{0}C overnight. 1-1.5 ml of bacterial culture was transferred into the sterile 2ml centrifuge tube. The tube was centrifuged at 8000rpm for 5 minutes at room temperature. The supernatant was discarded and the pellet was used for the DNA extraction.

4.11.7.2 Principle:

The cells are lysed with the enzyme Proteinase K and the nucleases are inactivated by chaotropic salt. The nucleic acids are bound to special silica fibers in the spin column tube. The cellular components contaminating the bound nucleic acid is removed in a series of rapid “wash and spin” steps. The elution releases the nucleic acids from the silica fiber.

4. 11.7.3 Extraction procedure

- All the steps were done at room temperature.
- The bacterial pellet was suspended in 200µl of phosphate buffered saline and vortexed for 30 seconds.
- Binding buffer of 400µl and 5µl of internal control template was added to the suspension.

- To the above suspension, 20µl of proteinase K was added.
- This was mixed immediately by inverting several times and incubated at 56°C for 15 minutes in a water bath.
- 200µl of Isopropanol was added and vortexed for 15 seconds.
- Entire sample was pipetted into a spin column.
- This was centrifuged for one minute at 13,000 rpm. Flow through was discarded and the spin column is transferred to a new centrifuge tube.
- 500µl of Wash buffer –I was added to the spin column.
- This was centrifuged for 60 seconds at 13,000 rpm. Flow through was discarded.
- 500µl of Wash buffer-II was added to the spin column.
- This was centrifuged for 60 seconds at 13,000 rpm and flow through was discarded and centrifuged for an additional one minute at 13,000 rpm to remove the residual ethanol.
- The spin column was transferred to a fresh 1.5ml micro centrifuge tube.
- 60µl of the Elution buffer (pre-warmed to 56˚C) was added to the centre of the spin column membrane. Care was taken not to touch the membrane with pipette tip.
- It was incubated for one minute at room temperature and centrifuged for one minute at 12,000 rpm.

The column was discarded and purified DNA was stored at -20°C.

Table:2 *bla*NDM reaction mix for samples:

S.No	Components	Volume
1.	Probe PCR Master Mix	10µl
2.	*bla* NDM-1 Primer Probe Mix	5µl
3.	Internal control Primer Probe Mix	5µl
4.	Purified DNA sample	5µl
5.	Total reaction volume	25µl

Table : 3 Primer and probe:

Primer	F : GTCTGGCAGCACACTTCCTA
	R : CGCCATCCCTGACGATCAAAC
Probe	TCTCGACATGCCGGGTTTCGG

Table:4. Amplification profile for blaNDM-1 gene:

	Step	**Time**	**Temp**
	Taq enzyme activation	15min	95^0 C
40cycles	Denaturation	20sec	95^0 C
	Annealing/ Data collection	20sec	56^0 C
	Extension	30sec	72^0 C

blaNDM-1 = FAM channel

Internal control = HEX channel

Internal control interpretation:

According to the protocols (assuming 100% extraction efficiency) the CT value is expected within 24-31 for the internal control. This may differ depending on the extraction efficiency, the quantity of elute added to the PCR reaction and the settings of machine. If the amplifying sample had high genome copy number, then the internal control may not produce an amplification plot. This can be interpreted as positive result rather invalidates it.

Table:5. Interpretation of results:

Test Sample	**Negative control**	**Internal control**	**Positive control**	**Interpretation**
Positive	Negative	Positive	Positive	**Positive**
Negative	Negative	Positive	Positive	**Negative**
Negative	Negative	Negative	Negative	**Repeat**
Positive	Positive	Positive	Positive	**Repeat**

5. RESULTS

5.1 The study group:

The study was conducted in the Department of Microbiology, Tirunelveli Medical College, over a period of 8months from December 2015-August 2016. 2514 samples received at a bacteriological laboratory were screened during the study period. Of which 425 isolates belonged to the *Enterobacteriaceae* family. Out of 425 isolates 195 isolates were multidrug resistant. These isolates were subjected to Ertapenem susceptibility testing by Kirby Bauer disc diffusion test. And then confirmed with Ertapenem E- test. Then these resistant isolates were subjected to MBL detection methods like Modified Hodge test, CDT, MBL E-test and carbapenemase inactivation test. Multiplex PCR is done to detect the presence of blaNDM-1. The sensitivity pattern to the antibiotics for the isolates and the other risk factors were analysed.

5.2 Statistical analysis:

All the results obtained were analysed statistically for their completeness, consistency and accuracy by the parameters like mean and percentages. The results were analysed by using IBM SPSS statistics 20. The statistical measures were completed with the help of the statistical software.

The p values were calculated using Chi square test, Fisher exact test and Mcnemer's test. The p values less than 0.05 was considered as significant.

5.3 Age and gender distribution of study isolates:

The study population were analysed based on age and sex. The result of the analysis are tabulated in Table 6

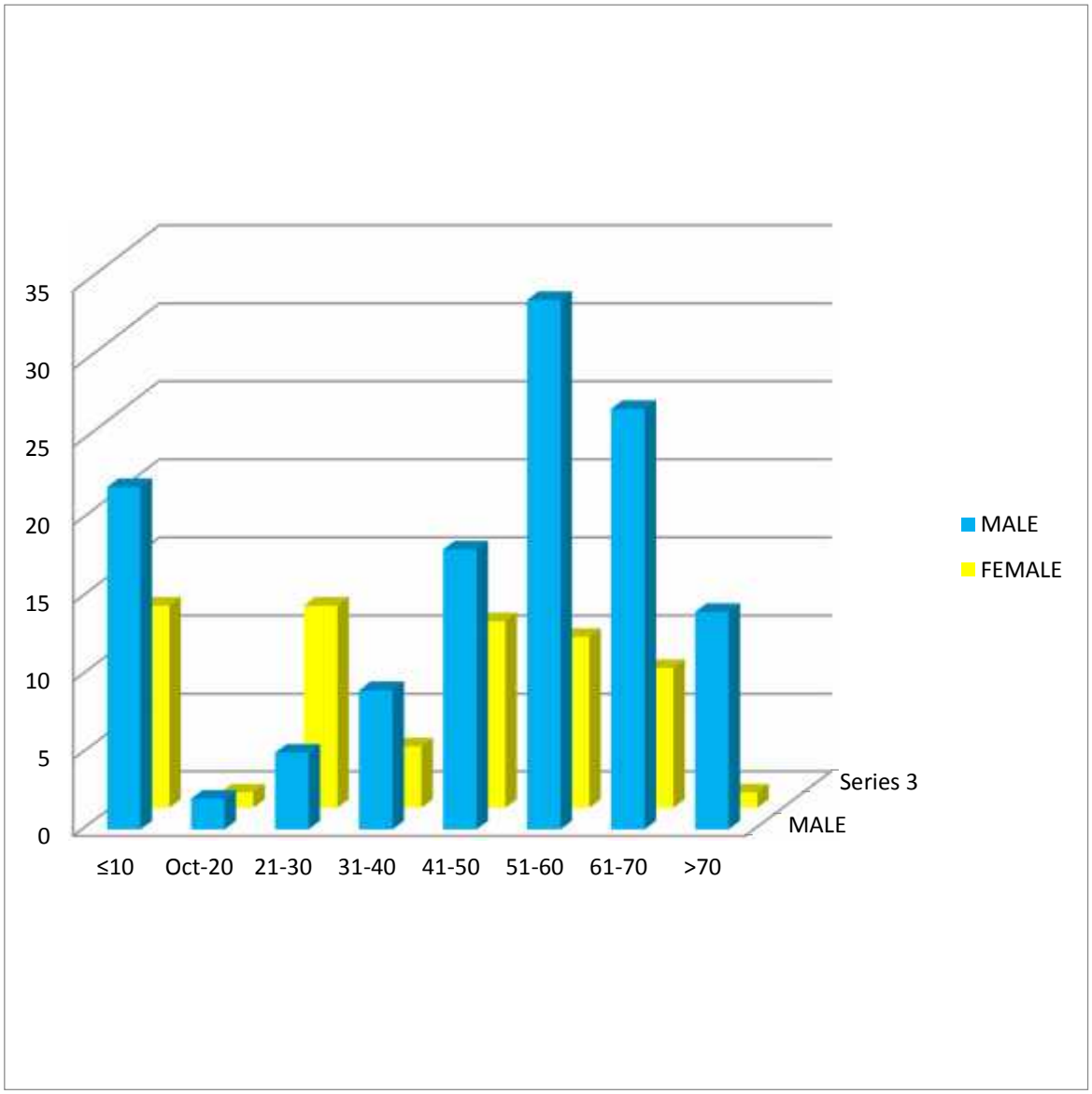
Table: 6 Age-Sex distribution of study isolates

AGE (YEARS)	MALE		FEMALE		TOTAL	
	NO	%	NO	%	NO	%
≤10	22	11.28%	13	6.67%	35	19.45%
11-20	2	1.02%	1	O.51%	3	1.53%
21-30	5	2.56%	13	6.67%	18	9.23%
31-40	9	4.61%	4	2.05%	13	6.67%
41-50	18	9.2%	12	6.15%	30	15.38%
51-60	34	17.43%	11	5.64%	45	23.07%
61-70	27	13.84%	9	4.61%	36	18.46%
≥70	14	7.74%	1	0.51%	15	7.69%
TOTAL	131	67%	64	33%	195	100%

The above table shows that among the 195 MDR *Enterobacteriaceae* isolates, 131 isolates (67%) were from males and the remaining 64 isolates (33%) were from

females. Male and female ratio was 2.07:1. The mean age was 45.63 years. (Table 6 and Figure.2)

Figure:2 Age and Sex distribution of study isolates



5.4 Distribution of MDR Enterobacteriaceae among clinical isolates

Table:7 Distribution of MDR *Enterobacteriaceae* among clinical isolates

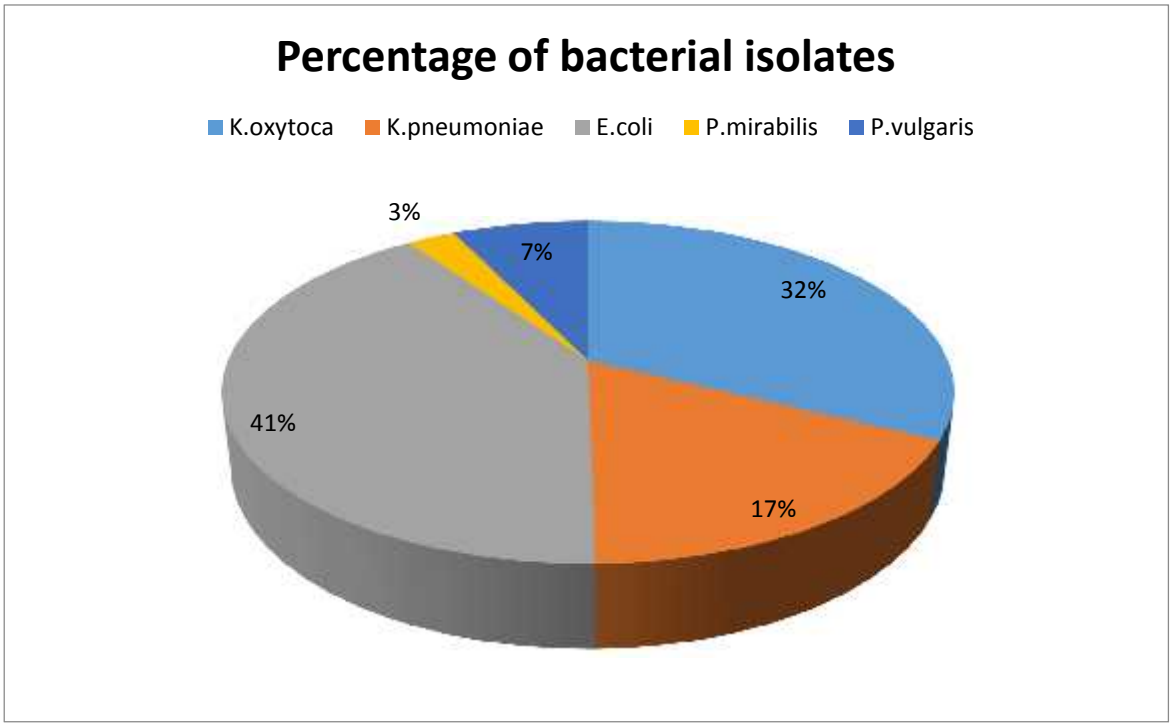
ORGANISM	No. of isolates (N=195)	PERCENTAGE(%)
Klebsiella oxytoca	63	32.30
Klebsiella pneumoniae	34	17.43
Escherichia coli	79	40.5
Proteus mirabilis	5	2.56
Proteus vulgaris	14	7.18

Out of 2514 samples screened during the study period, 425 were *Enterobacteriaceae* of which 195 samples were MDR *Enterobacteriaceae.* Distribution of organisms in this study group were *Klebsiella oxytoca* in 63 (32.3%) patients, *Klebsiella pneumoniae* in 34 (17.43%) of patients, Escherichia *coli* in 79 (40.5%) patients, *Proteus vulgaris* in 14 patients (7.2%)

and *Proteus mirabilis* in 5 (2.6%) patients. *Klebsiella* species was found to be a

most common multi drug resistant organism among clinical isolates. (Table :7 & figure:3)

Figure:3



5.5 Antibiotic susceptibility pattern of *MDR Enterobacteriaceae:*

Table: 8 Antibiotic resistances among MDR *Enterobacteriaceae*

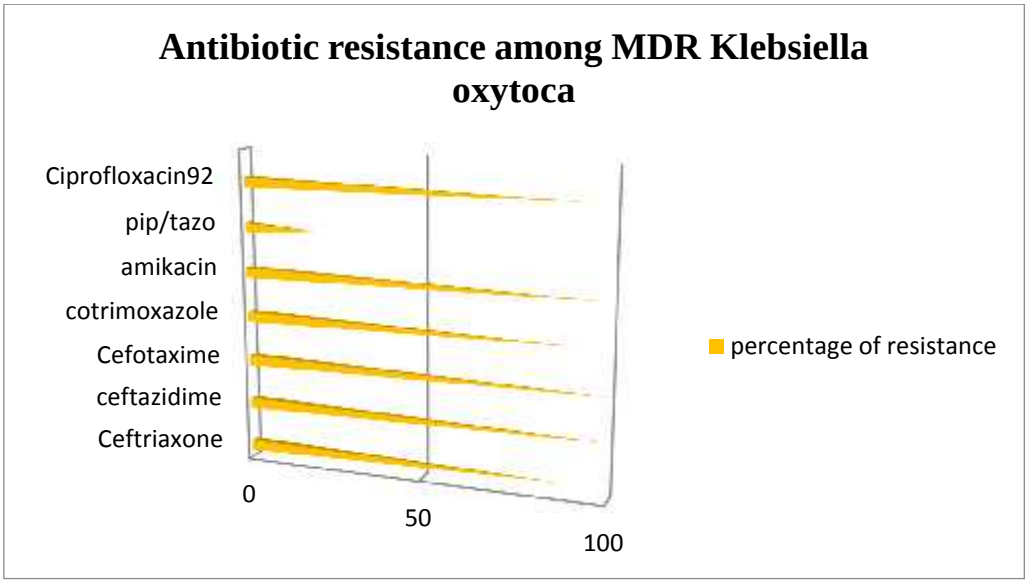
Antibiotics tested	***K.oxytoca* N = 63**		***K.pneumoniae* N =34**		***E.coli* N =79**		***P.vulgaris* N =14**		***P.mirabilis* N =5**	
	No	%	No	%	No	%	No	%	No	%
Ceftriaxone	55	87.3	34	100	79	100	14	100	5	100
Cefotaxime	63	100	34	100	79	100	14	100	5	100
Ceftazidime	63	100	34	100	79	100	14	100	5	100
Ciprofloxacin	58	92	31	91.1	76	96.2	12	85.7	4	80
Cotrimoxazole	57	90.4	32	94.1	77	97.5	13	92.8	5	100
Amikacin	61	96.8	32	94.1	77	97.5	11	78.6	5	100
Pip/Tazo	12	19.04	16	47	2	2.5	4	28.6	1	20

The above table shows the antibiotic resistance among MDR *Enterobacteriaceae.*

5.5.1 **Antibiotic resistance among MDR *Klebsiella oxytoca***:

MDR *Klebsiella oxytoca* showed 100% resistance to Cefotaxime and Ceftazidime, 87.3% resistance to Ceftriaxone, 96.8% resistance to amikacin,90.4% resistance to Cotrimoxazole and 19.4% resistance to Piperacillin /tazobactam (Table:8,Figure:4)

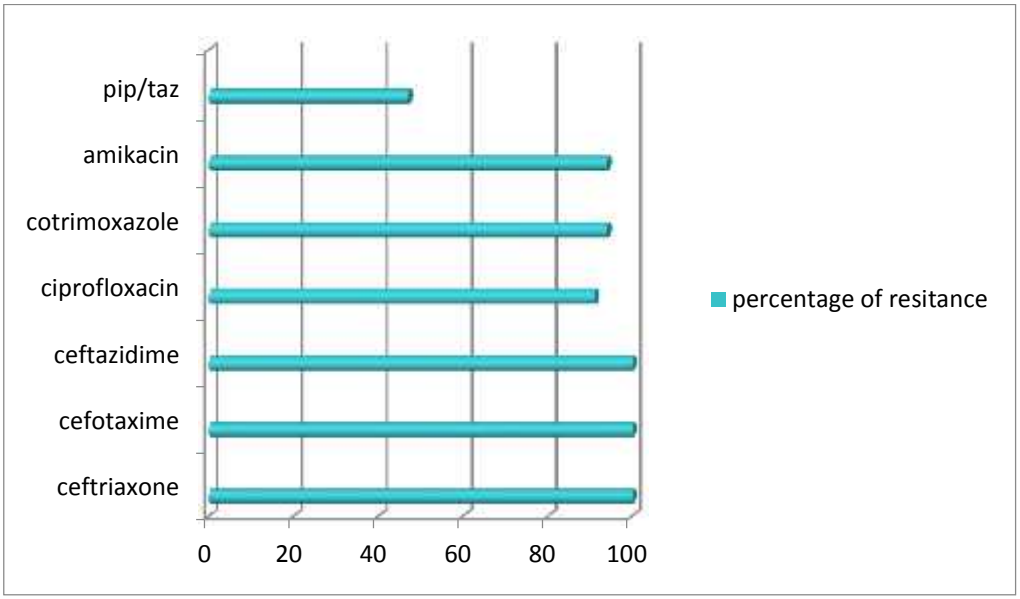
Figure:4



5.5.2 Antibiotic resistance among MDR Klebsiella pneumoniae:

Resistance pattern of MDR *klebsiella pneumonia* is shown in Figure :5. These isolates were 100% resistant to ceftazidime, ceftriazone and cefotaxime,91.1% resistance to ciprofloxacin,94.1% resistance to cotrimoxazole and amikacin,47% resistance to pip/taz. (Table:8)

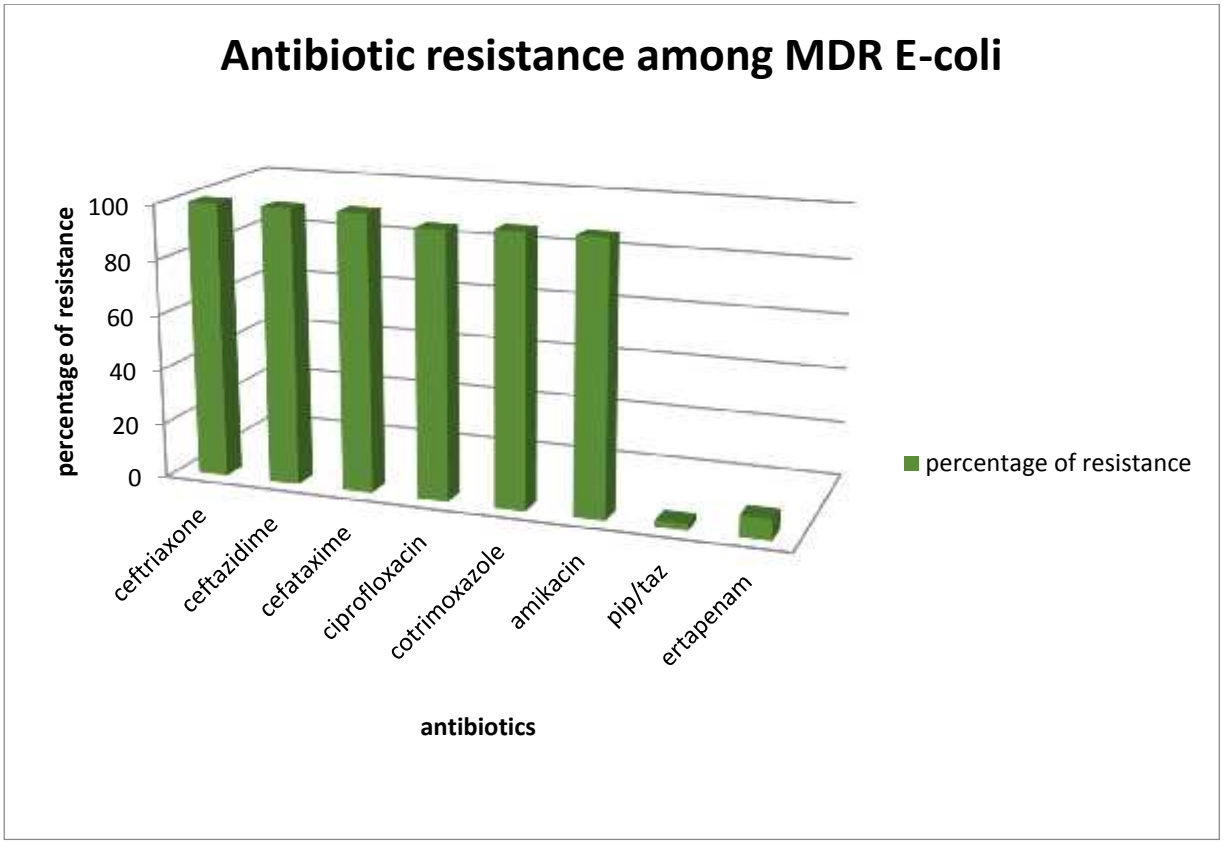
Figure:5 Antibiotic resistance among *Klebsiella pneumoniae*:



5.5.3 Antibiotic resistance among MDR E.coli:

Antibiotic resistance among MDR E-coli isolates are shown Figure:6.The isolates showed 100% resistance to ceftazidime,ceftriaxone and cefotaxime,97.5% resistance to cotrimoxazole and amikacin,96.2% resistance to ciprofloxacin and 2.5% resistance to piperacillin/tazobactam . (Table:8)

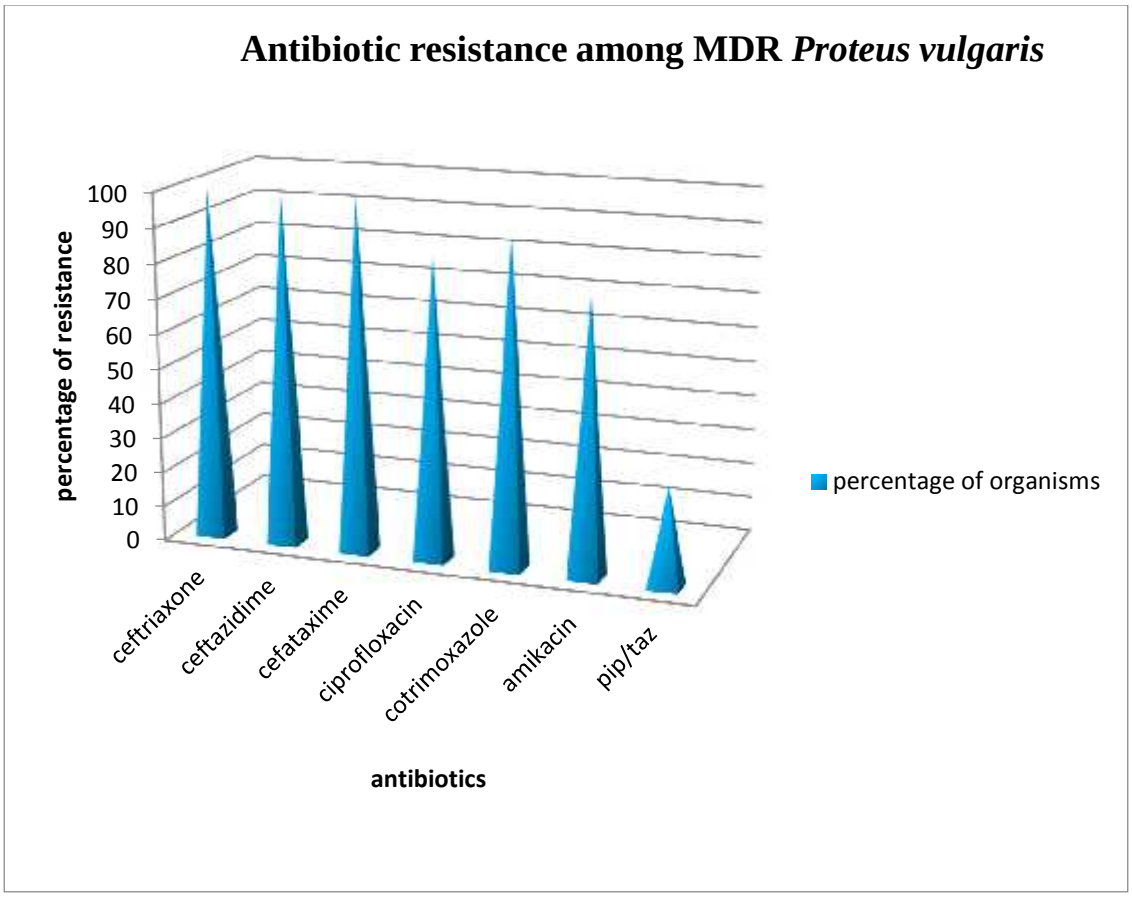
Figure:6



5.5.4 .Antibiotic resistance among MDR *Proteus vulgaris:*

The antibiotic resistance among MDR *Proteus vulgaris is shown in* Figure:7. These isolates showed 100% of resistance to ceftazidime, ceftriaxone and cefotaxime,85.7% resistance to ciprofloxacin,92.8% sensitivity to cotrimoxazole,78.6% resistance to amikacin and 28.6% resistance to piperacillin/tazobactam .(Table:8)

Figure:7

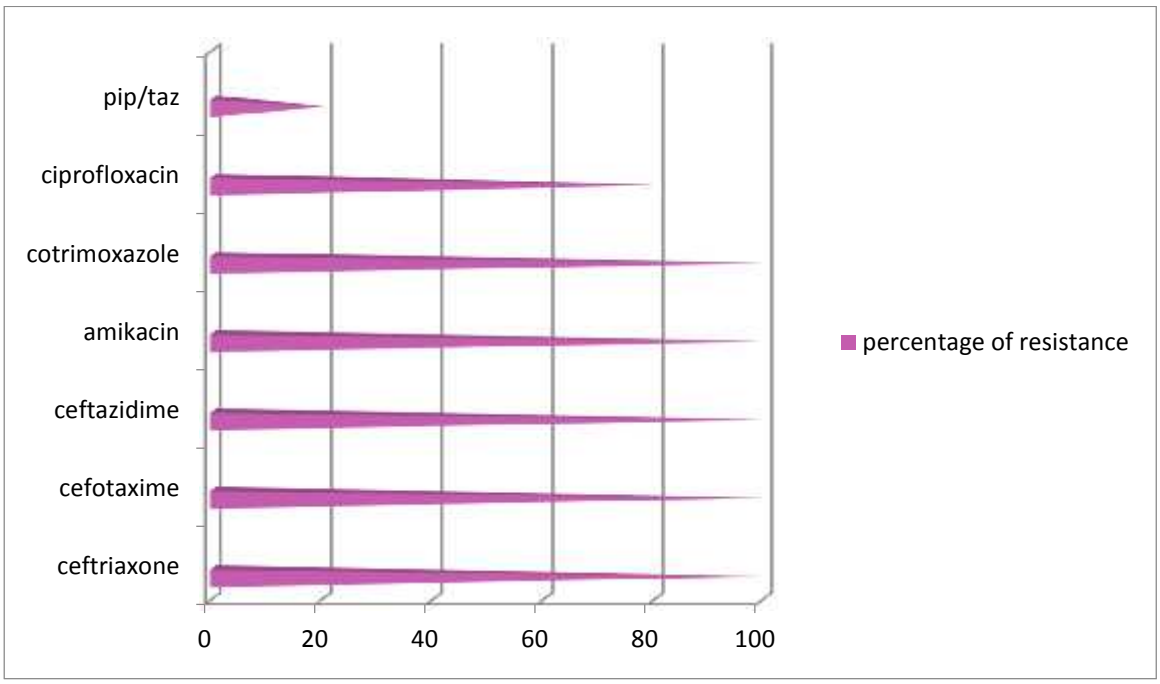


5.5.5 Antibiotic resistance among MDR *Proteus mirabilis:*

Antibiotic resistance among MDR *proteus mirabilis* shown in Figure :8. These isolates showed 100% resistance to ceftriaxone, ceftazidime, cefotaxime, amikacin and cotrimoxazole,20% resistance to ertapenem and piperacillin/tazobactam and 80% resistance to ciprofloxacin. (Table:8)

Figure:8

Antibiotic resistance among MDR *Proteus mirabilis*



5.6 Detection of Carbapenem resistance among study isolates:

Table:9a Detection of Carbapenem Resistance among study isolates

MDR Enterobacteriaceae	**Isolates [n=195]**
CRE	48 (25.6%)
CSE	147 (75.1%)

CRE: Carbapenem Resistant Enterobacteriaceae

CSE : Carbapenem Sensitive Enterobacteriaceae

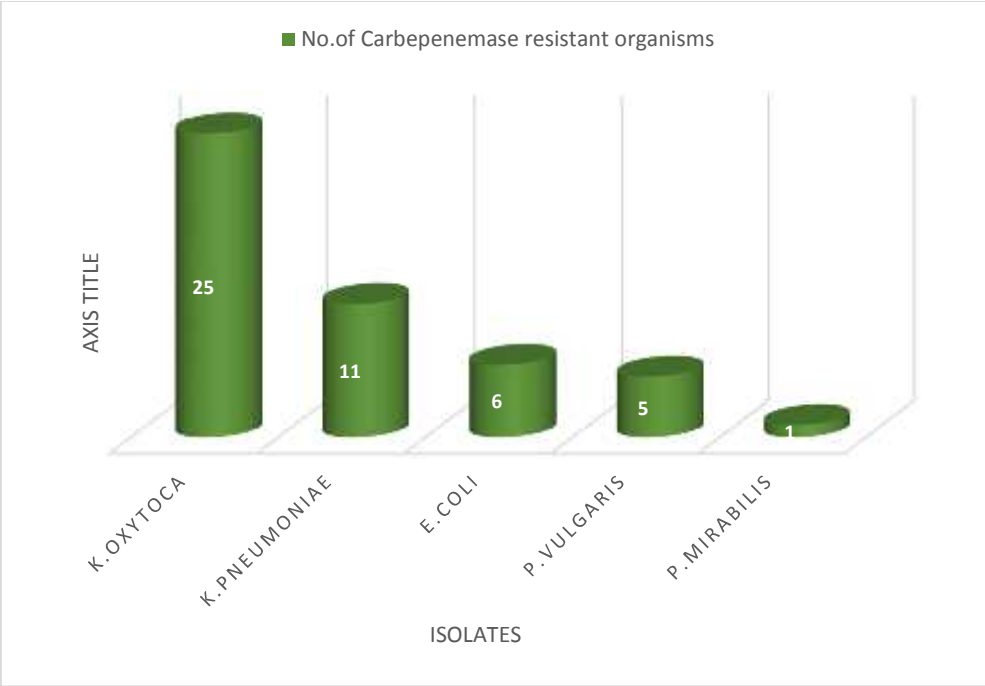
Table:9b Detection of Carbapenem Resistance among study isolates

Organisms N=48	*K.oxytoca*		*K.pneumoniae*		*E.coli*		*P. vulgaris*		*P. mirabilis*	
	No	%	No	%	No	%	No	%	No	%
Ertapenem	25	52.08%	11	22.91%	6	12.5%	5	10.41%	1	20.8%

The above table shows Carbepenem resistant Enterobacteriaceae among MDR *Enterobacteriaceae*. Out of 195 isolates 48(24.61%) were resistant and 147(75.1%) isolates were sensitive to ertapenem detected by disk diffusion test and Ertapenem E-test. (Table:9a)

Out of 48 Carbapenemase producers 25 isolates (52.08%),11 isolates (22.91%) were *K. pneumoniae*,6 isolates (12.5%) were *E. coli* 5 isolate (10.41%)s were *P. vulgaris* and one isolate (20.8%) was *P. mirabilis*. (Table:9b, Figure:9)

Figure:9 Detection of Carbepenemase resistance among study isolates:



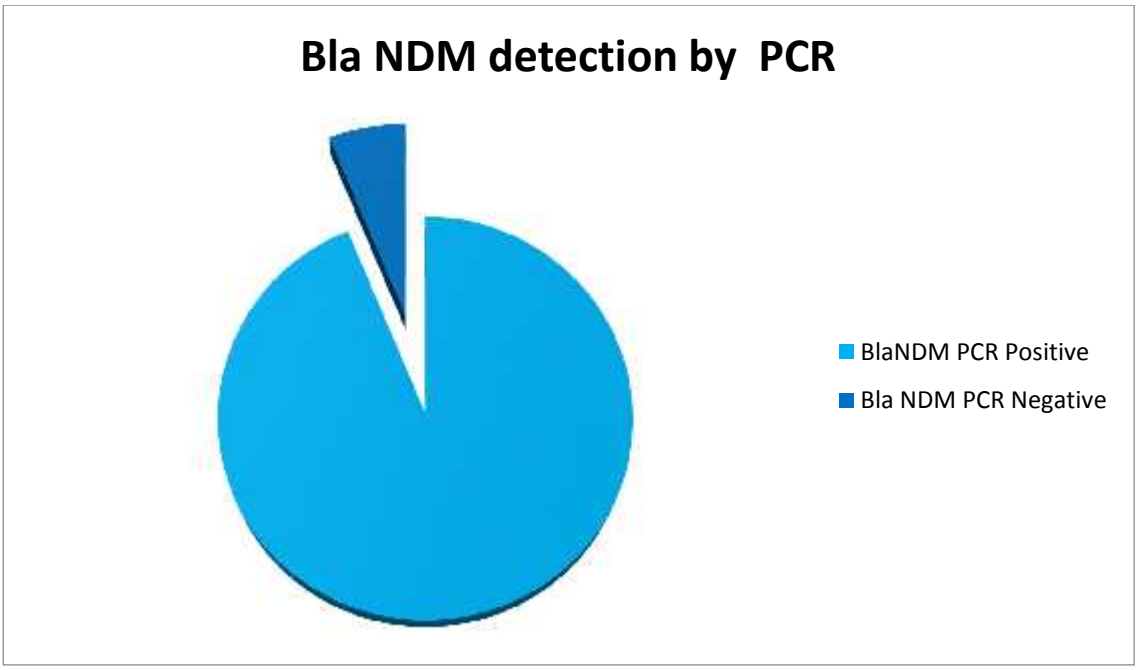
5.7 Detection of blaNDM-1 by PCR

Table:10 Detection of blaNDM-1 by PCR:

	Bla NDM PCR Positive	**Bla NDM PCR Negative**	**TOTAL**
CRE	**45**	**3**	**48**

The above table shows out of 48 carbpenem non susceptible isolates 45 isolates (93.75%) were positive for blaNDM-1 PCR. (Table : 10, Figure : 10)

Figure: 10



5.8 Correlation of bla NDM PCR and Modified hodge test:

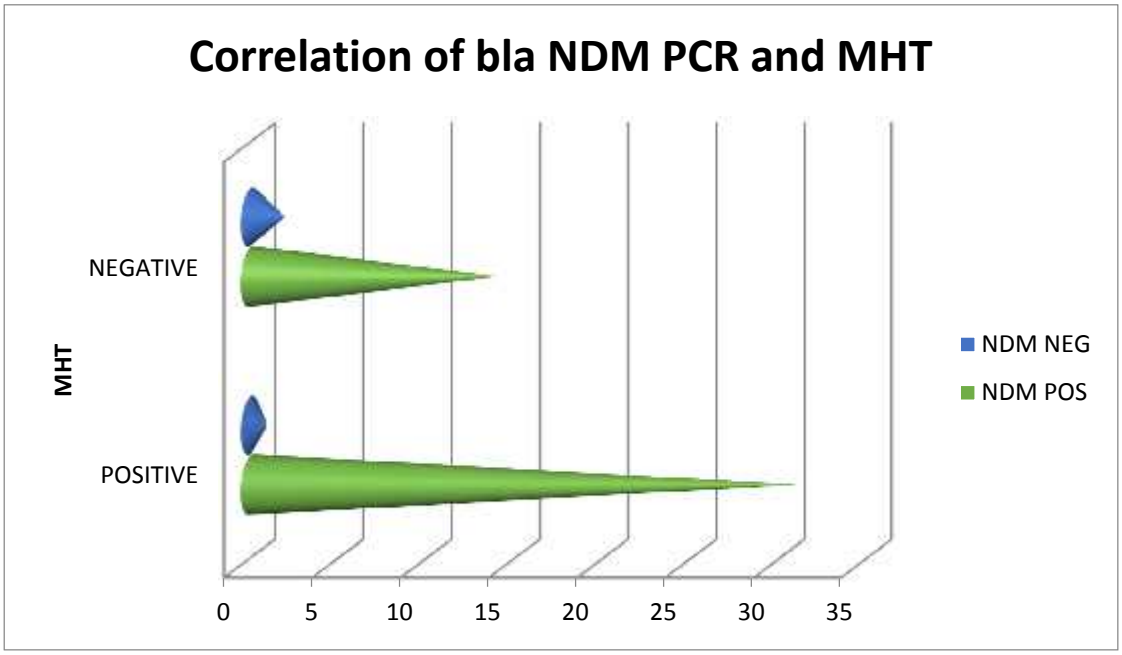
Table:11 Correlation of Modified Hodge test and blaNDM PCR

MHT	**bla NDM PCR**		**TOTAL**
	POSITIVE	**NEGATIVE**	
POSITIVE	31	1	32
NEGATIVE	14	2	16
TOTAL	45	3	48

The above table shows correlation of blaNDM PCR and MHT. Among the 48 CRE isolates, 31 isolates gave positive result with the MHT. The PCR detected the presence of MBL gene in 45 isolates. About 31 MHT positive isolates and 14 MHT negative isolates were PCR positive.

The sensitivity, specificity, positive predictive value and negative predictive value of MHT in the detection of MBL were 68%, 66.7%, 71.1% and 53.33% respectively. (Table: 11 and15b and Figure: 11). The kappa value denoting the measure of agreement is poor. (0.254).

Figure:11



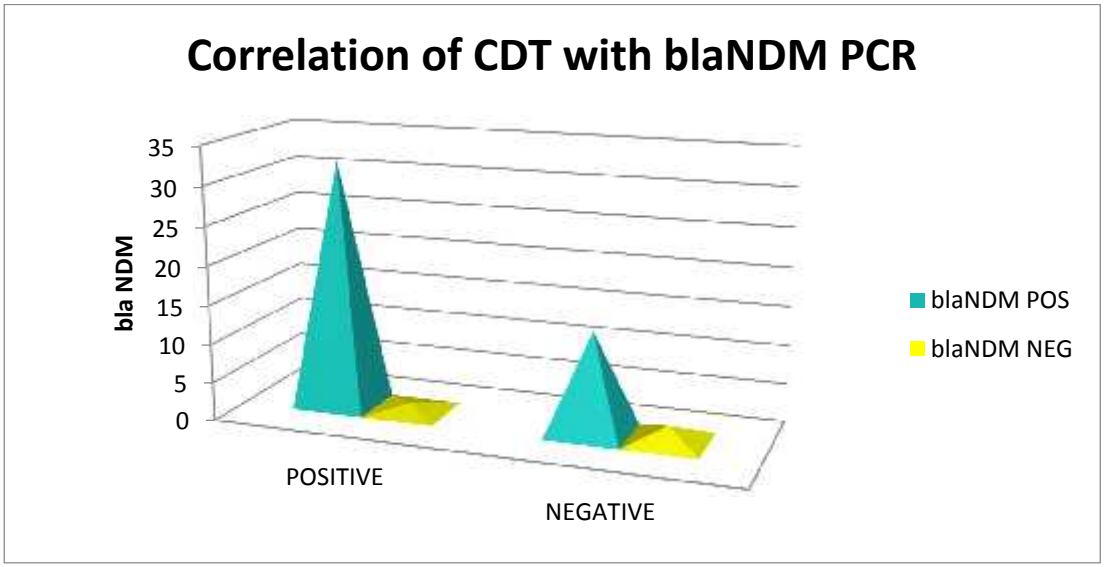
5.9 Correlation of CDT and blaNDM PCR:

Table:12 Correlation of CDT and bla NDM PCR:

CDT	bla NDM		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	32	1	33
NEGATIVE	13	2	15
TOTAL	45	3	48

The above table shows among the 48 CRE isolates, 32 isolates gave positive result with the CDT. The PCR detected the presence of MBL gene in 45 isolates. About 32 CDT positive isolates and 13 CDT negative isolates were PCR positive. The sensitivity, specificity, positive predictive value and negative predictive value of CDT in the detection of MBL were 71.1%, 66.7%, 97% and 66.7% respectively. (Table: 12 and 15b and Figure:12). The kappa value denoting the measure of agreement is poor (0.132)

Figure:12



5.10 Correlation of MBL Etest and blaNDM PCR for detection of MBL

Table:13 Correlation of MBL E-test with bla NDM PCR

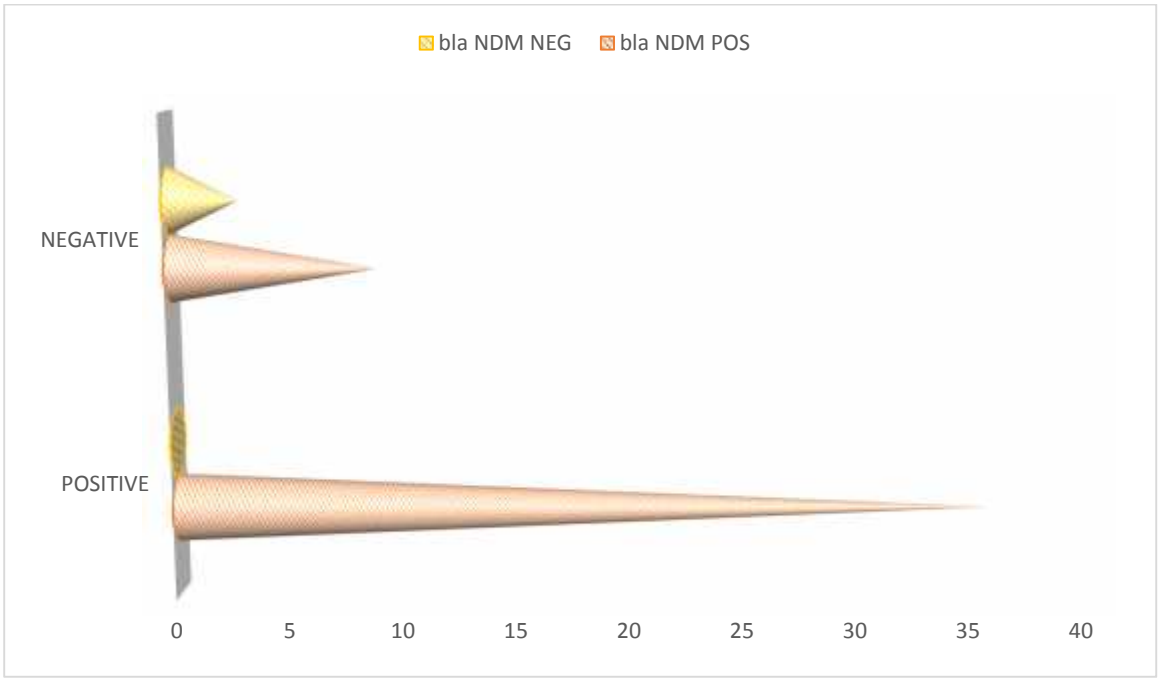
E-TEST	bla NDM		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	36	-	36
NEGATIVE	9	3	12
TOTAL	45	3	48

The above table shows among the 48 CRE isolates, 36 isolates gave positive result with the MBL E -test. The PCR detected the presence of MBL gene in 45 isolates.

About 36 MBL E-test positive isolates and 9 MBL E-test negative isolates were PCR positive.

The sensitivity, specificity, positive predictive value and negative predictive value of MBL E test in the detection of MBL were 76.5%, 100%, 80% and 100% respectively. (Table: 13 and 15b and figure 13). The kappa value denoting the measure of agreement was poor (0.333).

Figure:13 Correlation of MBL E-test with bla NDM PCR



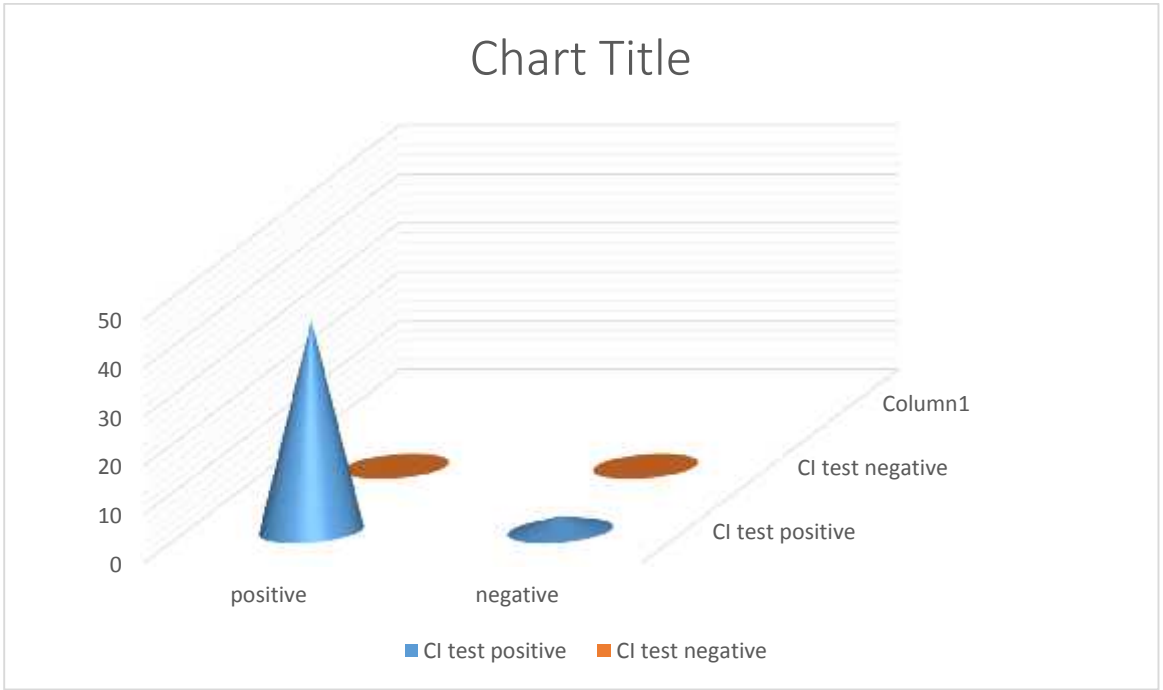
5.11 Correlation of carbapenemase Inactivation test and PCR

Table: 14 Correlation of Carbapenemase inactivation test with bla NDM PCR

BlaNDM PCR	CI test		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	42	0	42
NEGATIVE	3	3	6
TOTAL	45	3	48

Figure:14

Correlation of Carbapenemase inactivation test and blaNDM-1 PCR



The above table shows among the 48 CRE isolates, 46 isolates gave positive result with carbapenemase inactivation (CIM) test. The PCR detected the presence of MBL gene in 45 isolates. About 43 MBL CIM test positive isolates and 2 CIM test negative isolates were PCR positive.

The sensitivity, specificity, positive predictive value and negative predictive value of CIM test in the detection of MBL were 100%, 33.3%, 100% and 100% respectively. The kappa value denoting the measure of agreement is good (0.636) (Table: 14 and 15b and figure 14).

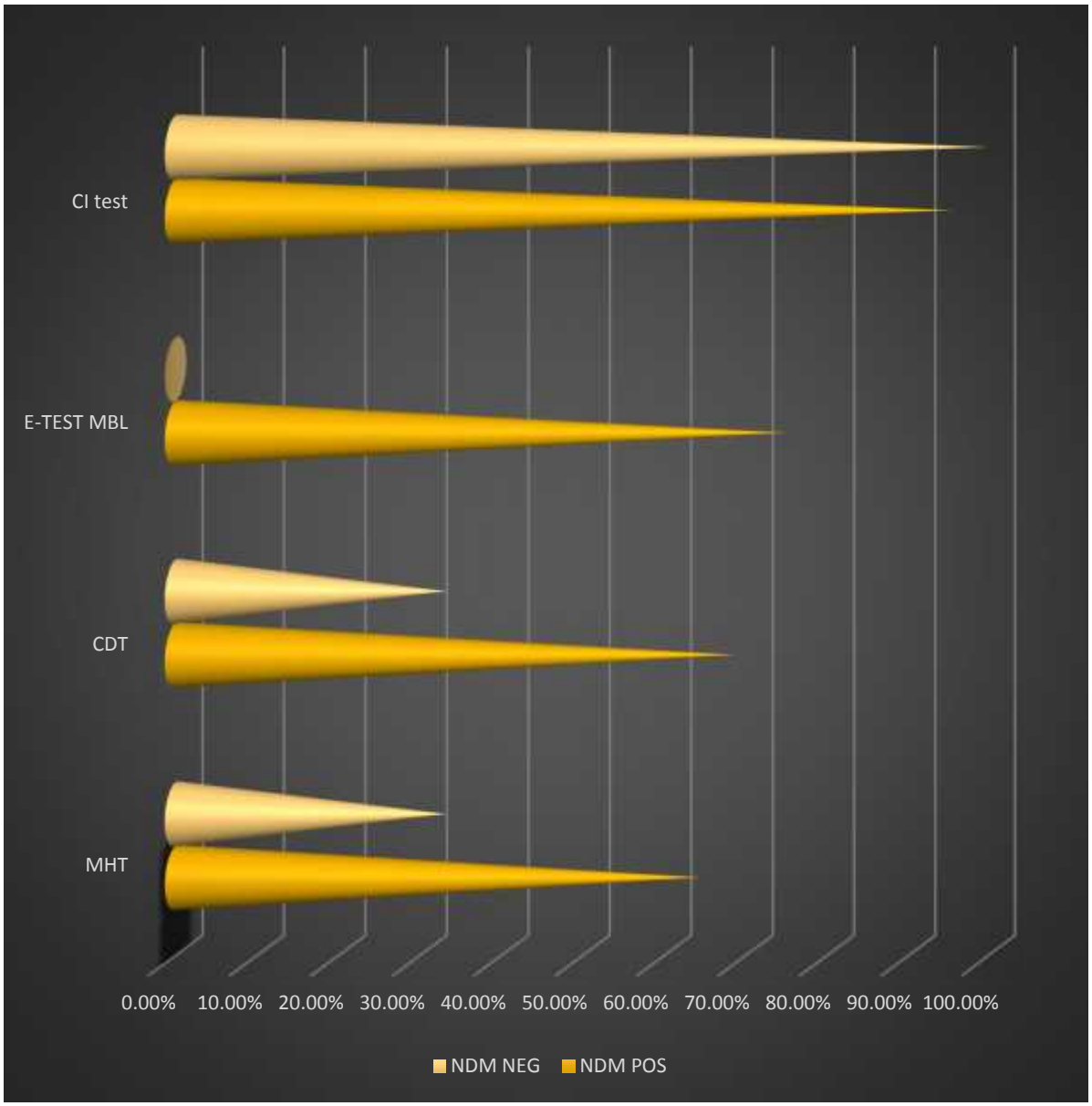
5.12 Comparison of MBL detection phenotypic methods with PCR:

The below table shows that 48 Ertapenem non susceptible, which were evaluated for the production of MBL by various phenotypic methods like CDT, MHT, MBL E test and carbapenemase inactivation test. Among them, 31isolates showed MBL production by MHT and 32 were positive with the CDT.The MBL E test detected about 36 isolates and CIM detected 46 isolates which were considered positive for MBL production. (Table:15 and Fig:15)

Table:15 a Comparison of MBL detection phenotypic methods with PCR:

METHODS	MBL PRODUCERS			
	Bla NDM PCR Positive N=45	**percentage**	**Bla NDM PCR Negative N=3**	**percentage**
MHT	31	64.58%	1	33.3%
CDT	32	68.75%	1	33.3%
E-TEST	36	75%	0	0%
Carbapenemase inactivation test	43	95.6	3	100%

Figure: 15 a Comparison of MBL detection phenotypic methods with PCR:



5.13 Evaluation of Phenotypic methods of MBL detection:

Table:15 b Evaluation of Phenotypic methods of MBL detection

Method	Sensitivity	Speificity	Positive predictive value	Negative predictive value
MHT	68%	66.7%	71.1%	53.33%
CDT	71.1%	66.7%	97%	66.7%
MBL E-test	76.5%	100%	80%	100%
Carbapenemase inactivation test	100%	33.3%	100%	100%

The above table shows sensitivity,specificity,positive predictive value and negative predictive value of phenotypic tests.

5.14 Table:16 Prevalence of blaNDM-1 among study isolates:

Enterobacteriaceae	**No of isolates N= 425**	**Percentage of isolates (100%)**
MDR *Enterobacteriaceae*	195	45.88%
Carbapenem resistant *Enterobacteriaceae*	48	11.29%
Bla NDM-1 positive isolates	45	10.6%

The above table shows out of 425 *Enterobacteriaceae* isolates 195 isolates(45.88%) were Multidrug resistant,among this 48 isolates (11.29%) were resistant to carbepenems and from these isolates blaNDM-1 was detected in 45(10.6%) isolates. (Table:16)

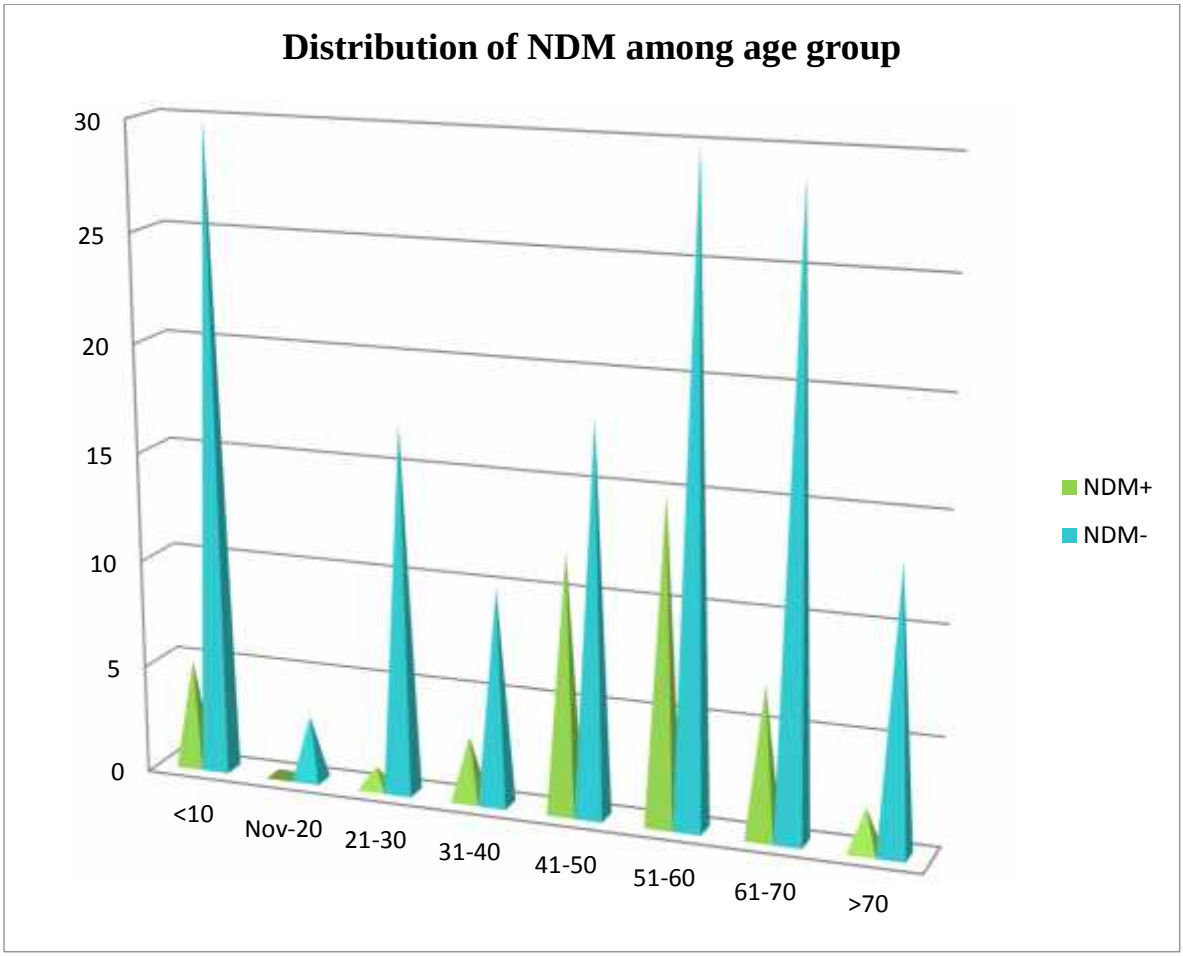
5.15 Table1-17 Distribution **of blaNDM-1 among age group:**

Age in yrs	**NDM positive N=45**	**Percentage**	**NDM Negative N=150**	**%**
< 10	5	11	30	20
11-20	0	0	3	1.58
21-30	1	2.2	17	11.3
31-40	3	6.7	10	6.67
41-50	12	26.7	18	12
51-60	15	33.3	30	20
61-70	7	15.6	29	19.3
>70	2	4.5	13	8.7
Total	45	100	150	100

The above table shows distribution of blaNDM positive isolates by age . In the age group of below 10 years, among 35 isolates five isolates (11%) were positive for blaNDM.No isolates were positive between 11-20 yrs. One isolate (2.2%) was positive between 21-30 years among 18 isolates. Three isolates were positive between 31-40 years among 13 isolates. 12 isolates were positive between 41-50 yrs among 30 isolates.15 isolates were positive between 51-60 yrs among 45 isolates and seven isolates were positive among 61-70 yrs group.2 isolates were positive out of 15 isolates

belonged to more than 70 yrs group from males and two isolates (50%) were from females. Mean age for blaNDM positive isolates was 45.73years. (Table :17, Figure:16)

Figure: 16



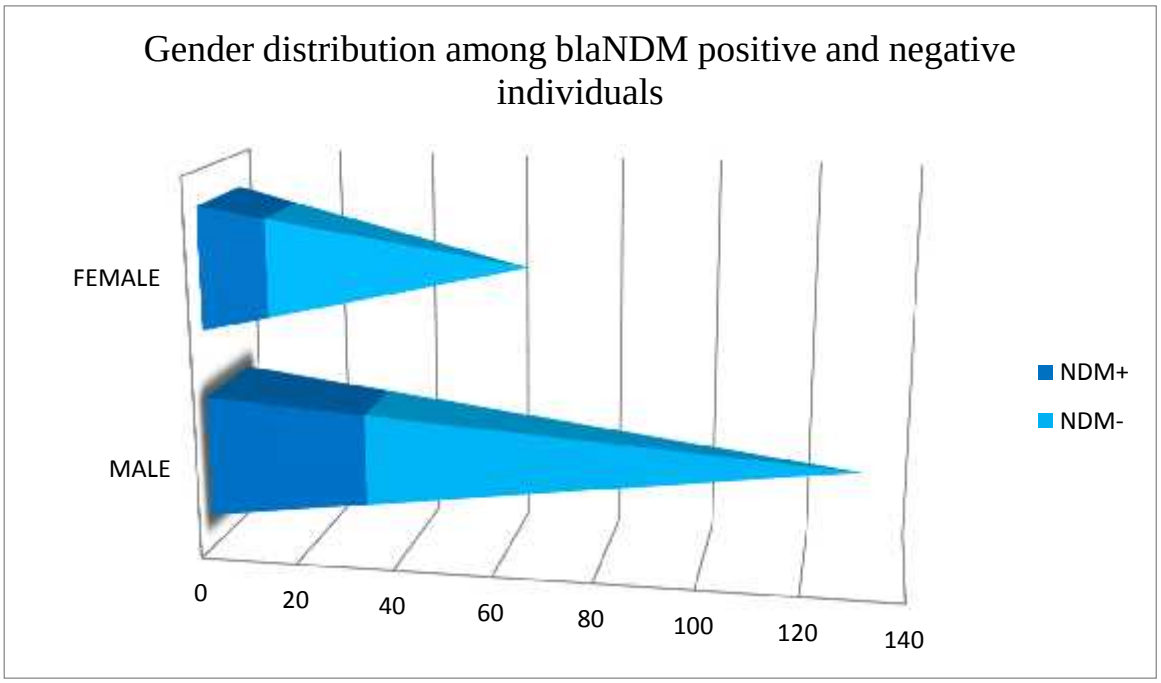
5.16 SEX RATIO:

Table: 18 Distribution of blaNDM among gender:

SEX	NDM positive	Percentage	NDM Negative	Percentage
MALE	32	71	99	66
FEMALE	13	29	51	34
TOTAL	45	100	150	100

The above table shows sex distribution of blaNDM positive Isolates. Male Female ratio is 2.4:1 among positive isolates. (Table: 18 and Figure: 17)

Figure:17



7

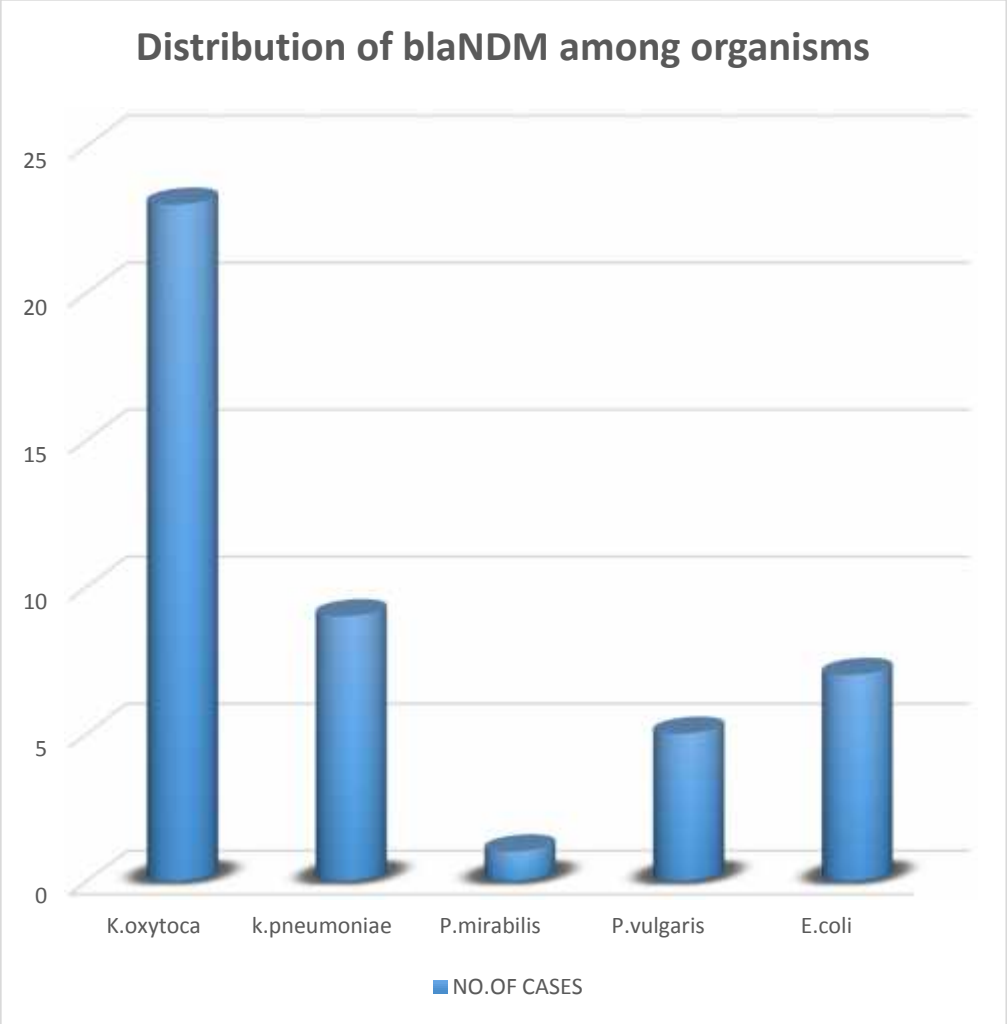
5.17 Bla NDM-1 positive pathogens:

The below table shows that out of 48 carbapenamase resistant isolates 45 were positive for blaNDM PCR.The blaNDM positive organisms were *Klebsiella oxytoca* in 23(51%) patients, Klebsiella *pneumoniae* in 9(20%) patients *E.coli* in 7(15.6%) patients *Proteus mirabilis* in one patient(2.22%)and *Proteus vulgaris* in 5(11%) patients.(Table-19 & figure 18)

Table:19 Distribution of blaNDM-1 among organisms:

Organisms	No of cases	%
Kleb.oxytoca	23	51.1
Kleb.pneumoniae	9	20
Prot.mirabilis	1	2.22
Prot.vulgaris	5	11.1
E.coli	7	15.6

Figure:18


Distribution of blaNDM among organisms
25
20
15
10
5
0
K.oxytoca
k.pneumoniae
P.mirabilis
P.vulgaris
E.coli
NO.OF CASES

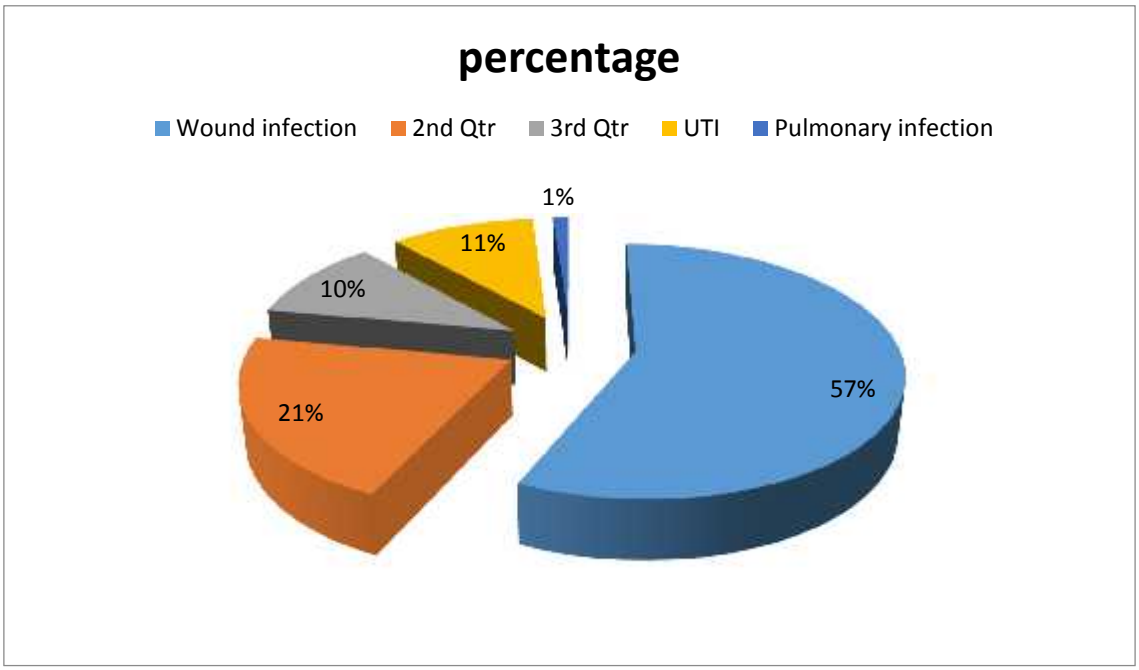
5.18 Infection wise distribution of blaNDM-1 isolates;

The below table shows that majority of the NDM infections were associated with wound infection about 27 isolates (60%) followed by UTI in 10 isolates (22.2%) and Sepsis in 5 (11.1%) isolates, Pulmonary infection in 3 isolates (6.38%).(Table 21& figure:18)

Table:20 **Infections wise distribution of blaNDM-1 positive Isolates**

Infections	BlaNDM-1positive isolates	
	NO	%
Wound infection	27	60
UTI	10	22.2
Sepsis	5	11.1
Pulmonary infection	3	6.38

Figure:19



5.19 Distribution of blaNDM from different wards:

Figure:20

Distribution of bla NDM isolates from different wards:

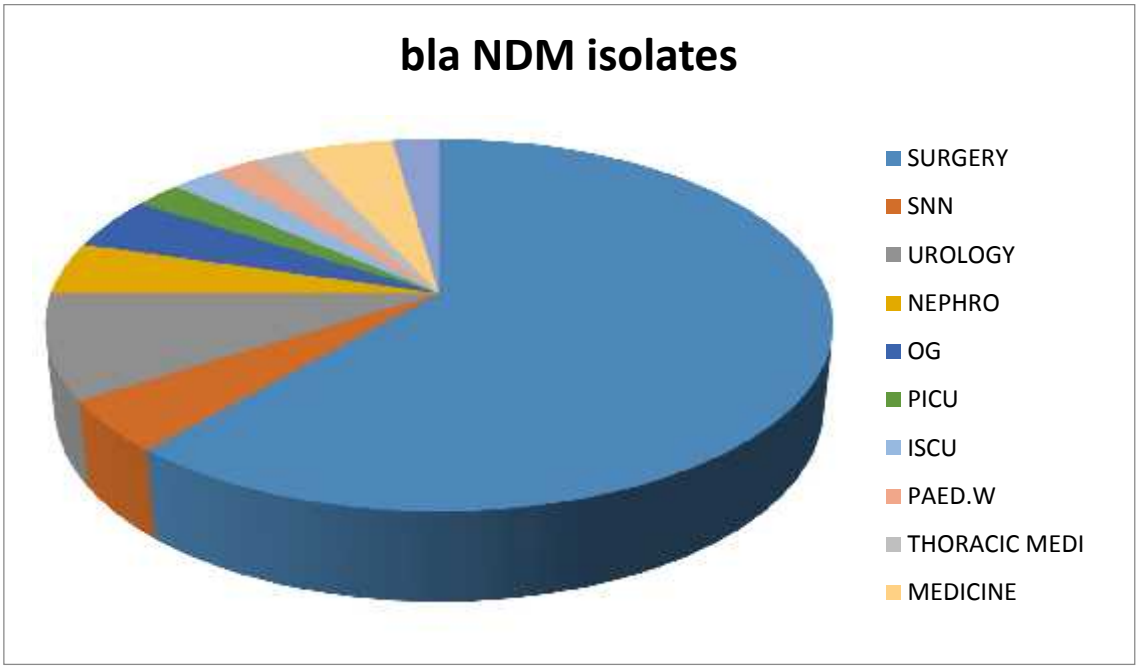


Table:21 Ward wise distribution of NDM isolates :

WARDS	blaNDM isolates	
	No	**%**
SURGERY	27	60
SNN	2	4.4
UROLOGY	4	8.9
NEPHRO	2	4.4
OG	2	4.4
PICU	1	2.22
ISCU	1	2.22
IMCU	1	2.22
PAED.WARD	1	2.22
THOR.MED	1	2.22
MEDICINE	2	4.4
CAS.WARD	1	2.22

Above table shows distribution of bla NDM positive organisms among different wards. Out of 45 positive patients majority(60%) were from surgery ward.(Figure 15 and Table :21)

5.20 Analysis of risk factors:

5.20.1 **Relation of bla NDM to duration of hospital stay :**

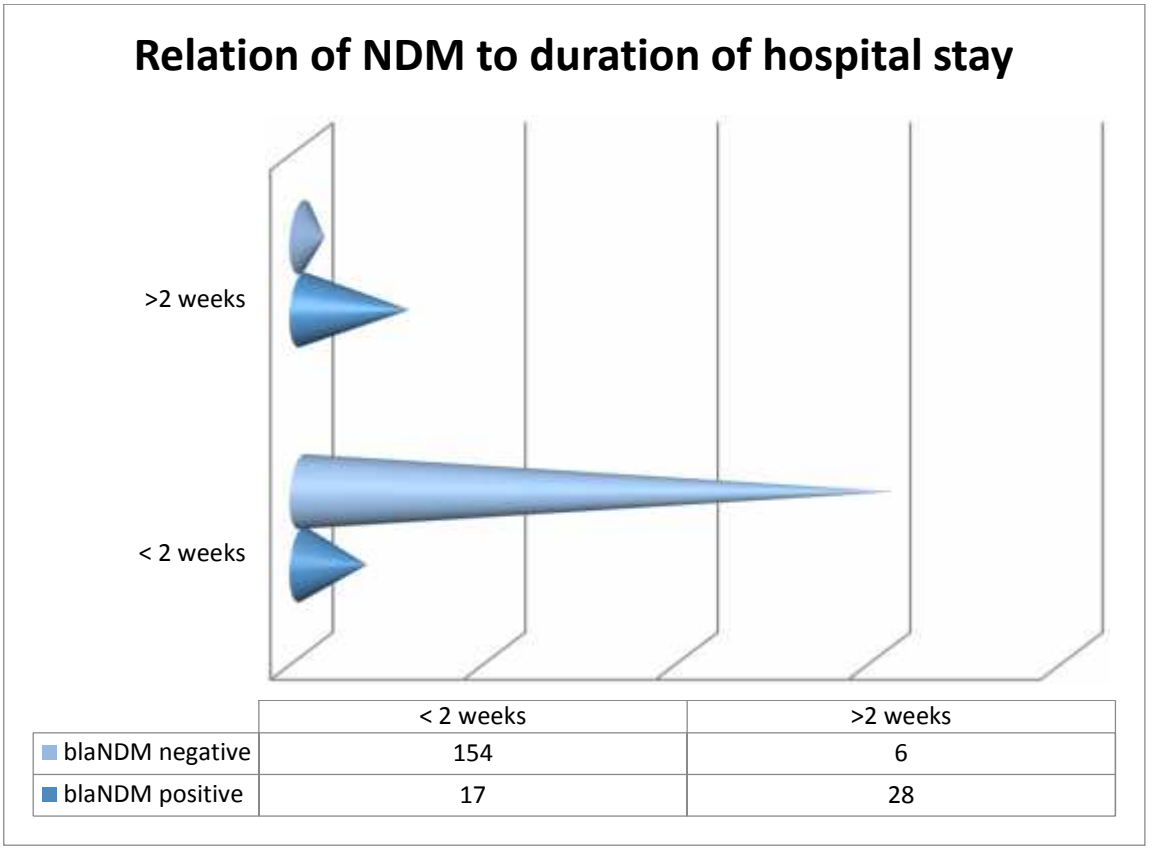
Table:23 and figure :21 shows out of 45 blaNDM positive patients 28were admitted in the hospital for more than 2weeks and 17 patients were admitted in the hospital for less than two weeks. The association of blaNDM positive isolates with the duration of stay in hospital was statistically significant.

$P < 0.05$

Table:22 **Relation with duration of hospital stay:**

DURATION	**Bla NDM-1**			
	POSITIVE		**NEGATIVE**	
<2 WEEKS	17	37.78	154	96.25
>2 WEEKS	28	62.2	6	3.75
TOTAL	45	100	160	100

Figure:21



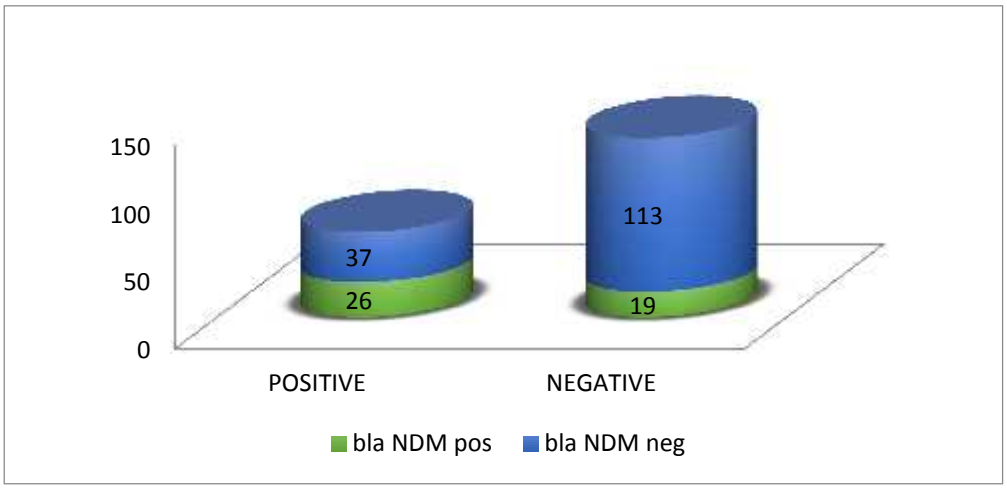
5.20.2 Relation of blaNDM to Diabetes Mellitus:

Table:23 Relation with Diabetes mellitus

DM	bla NDM positive		bla NDM negative		TOTAL
	NO	%	NO	%	
POSITIVE	26	57.8	37	24.7	63
NEGATIVE	19	42.2	113	75.3	132
TOTAL	45	100	150	100	150

Table:23 shows relation of blaNDM to diabetes mellitus .Out of 45 positive patients 26 patients have diabetes mellitus and out of 150 negative patients 37 patients have diabetes mellits. This association is statistically significant $P < 0.05$.

Figure:22 Relation of blaNDM positivity and diabetes mellitus:



5.20.3 Relation of blaNDM to exposure of carbapenems:

Table:24 Relation of blaNDM and carbapenem exposure

Carbapenems administered	**Bla NDM positive**		**Bla NDM Negative**	
	No	%	No	%
Yes	23	51.1	16	10.7
No	22	48.09	134	89.3
Total	45	100	150	100

Figure:22

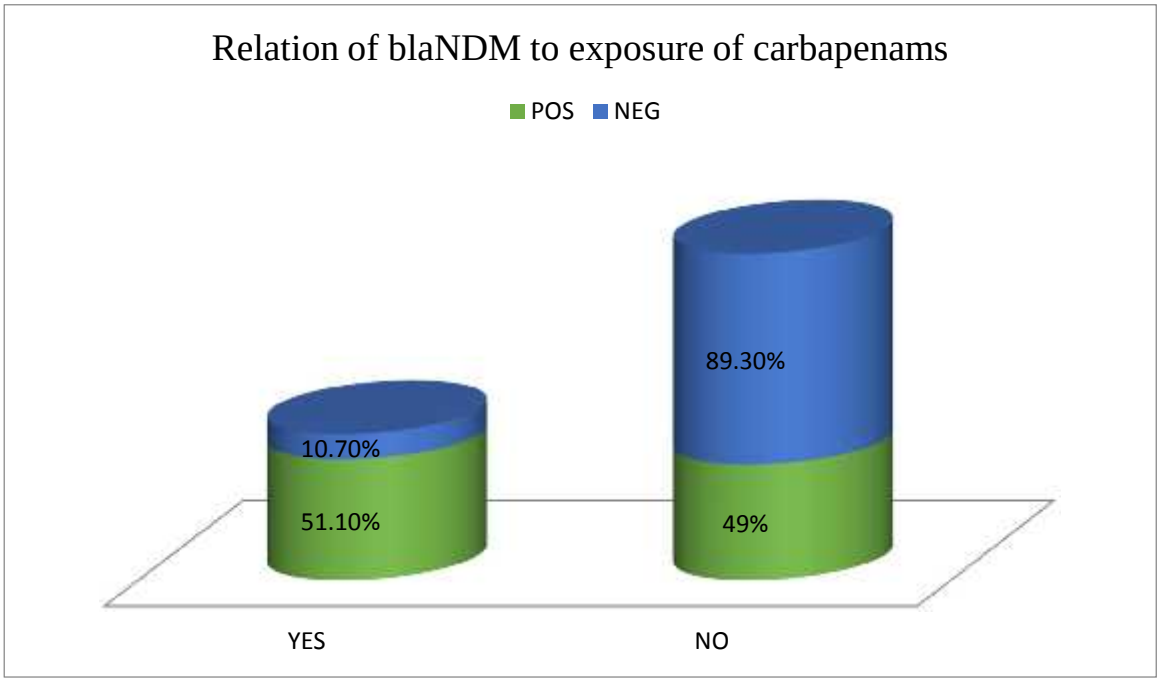


Table 24 and figure :22 shows relation of carbapenam exposure to blaNDM positive patients.Out of 45 patients 23 were exposed to carbapenams. This association is statistically significant P<0.05

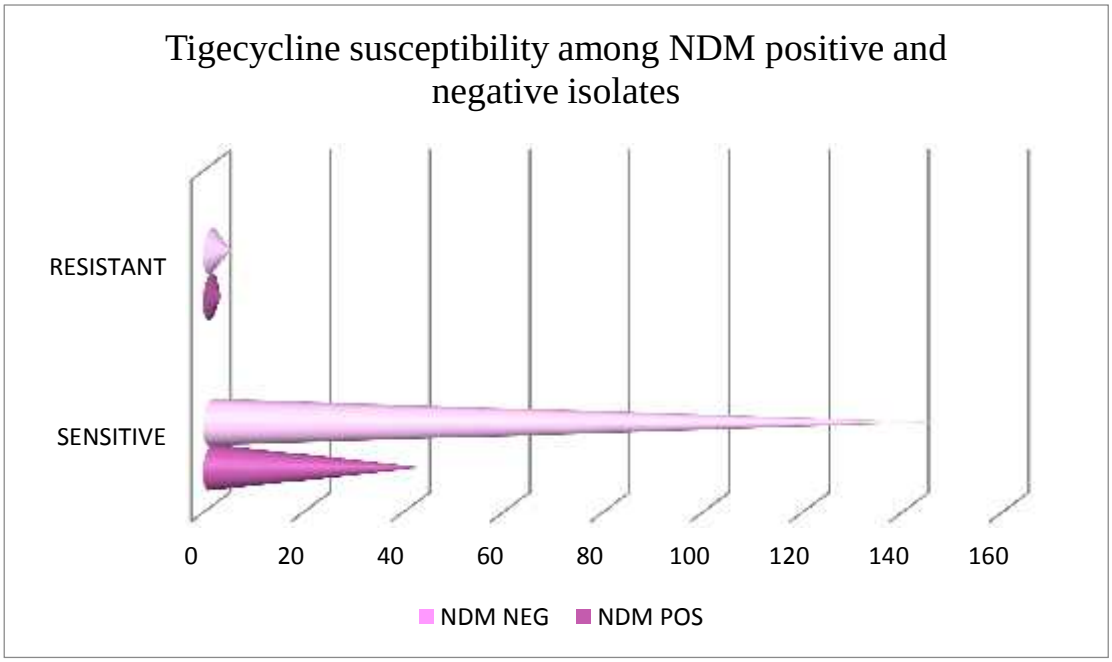
5.21 Comparison of Tigecyline susceptibility among blaNDM positive and negative patients.

Table:25 **Tigecycline susceptibility**

Tigecycline sensitivity	**Bla NDM PCR**			
	Positive		**Negative**	
Sensitive	43	95.6%	146	97.33%
Resistant	2	4.4%	4	2.7%
Total	45	100%	150	100

Table 25 and figure 23 shows tigecycline susceptibility among blaNDM-1 positive and bla NDM negative patients. Out of 45 bla NDM positive patients two (4.4%) were resistant for tigecycline compared to four (2.7%) patients among blaNDM negative patients

Figure::24



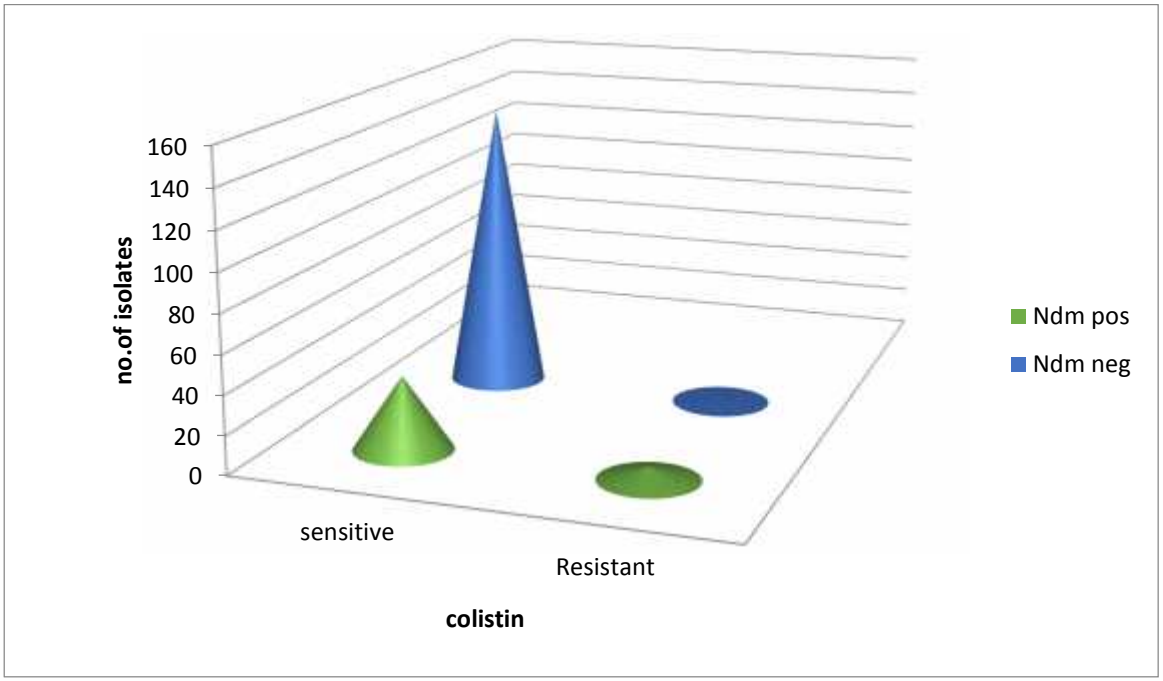
5.22 Comparison of colistin susceptibility among blaNDM positive and blaNDM negative isolates

Table:26 **Colistin susceptibility**

Colistin susceptibility	Bla NDM			
	POSITIVE		NEGATIVE	
Sensitive	33	73.3%	136	90.7%
Resistant	12	26.7%	14	9.3%
Total	45	100%	150	100%

The above table shows that among the 195 MDR *Enterobacteriaceae* 26 patients were resistant to colistin in which 12(15.6%) patients were blaNDM positive.(Table:27,fig:25)

Figure:25 Colistin susceptibility



6.DISCUSSION

The emergence of infections due to carbapenem resistant Enterobacteriaceae represents a major public health concern. They are nosocomial and community acquired pathogens associated with high mortality rates and they have the potential to disseminate extensively. So early detection and implementation of interventions are required to control these organisms in health care facilities.

6.1 Prevalence:

Prevalence of blaNDM-1 among *Enterobacteriaceae* in this study was 10.6% and among CRE it was 93.75%.

Several studies from India reported that, the prevalence of NDM-1 producers among CRE was between 31.2% to 91.6% [10.84.89], similar to our study.

Mohan et al reported 84.3% of blaNDM-1 among CRE,this report was concordant with our report[86].**Ramesh** et al reported comparable result of 12.8% among GNB[87]. Similar study conducted by **Karthikeyan** et al found that out of 47 (24%) CRE isolates 26(55.31%) were positive for NDM-1. **Parveen** et al reported the prevalence of *bla* NDM-1 was 70.6% among carbapenemase producing strains. **Teo et al** reported that out of Fifty-two carbapenem 23% were positive for bla NDM-1.

6.2 Distribution of blaNDM -1 among age and sex:

Out of 195 MDR positive isolates 131(67%) were males and 64 (33%) were females. Male to female ratio was 2.1:1. Mean age was 45.63 years. The mean age for blaNDM positive isolates was 45.73 years and male to female ratio was about

2.4:1.Rate of infection was 32/131(24.4%) in males which was higher than females.(20.3%) .

But **Karthigeyan** et,al in his study reported that the age range 4-66 yrs with a mean age of 36 years and female to male ratio about 2 to 1.

6.3 Distribution of bla NDM-1 among organisms:

In our study out of 45 blaNDM-1 positive isolates ,most common organisms were *Klebsiella oxytoca* accounted for 23 isolates(51.15%) followed by *Klebsiella pneumoniae* n = 9(20%) , *E.coli* n= 7(15.6%) , *proteus vulgaris* n=5(11.1%) and *Proteus mirabilis* n=1(2.22%)

Bhaskar et al reported that out of 66 *bla* NDM-1 positive isolates, 40 isolates were *Klebsiella* spp, 6 isolaes from *E.coli* and 20 isolates from *Enterobacter* spp similar to our study report.

Study reports from **yong et.al, Kumarasamy et, al. Struelens et.al**, **Jovcic et.al& B. Karthikeyan K et.al,** showed that presence of NDM-1 has reported from *Enterobacteriaceae* as well as *Pseudomonas* and *Acinetobacter* species. [10,90,91,38]

Kumarasamy KK et,al in his study showed that was *Escherichia coli* and *Klebsiella pneumonia* species accounts for majority of the production of NDM compared to other *Enterobacteriaceae.*[10,84]

Lascols et al., in 2011 reported that the blaNDM-1 gene was detected in a range of bacterial strains belongs to the family *Enterobacteriaceae* including *Escherichia coli, K. pneumoniae, Enterobacter cloacae, Providencia rettgeri and M. morganii*

6.4 Distribution of blaNDM-1 among different specimens:

In our study distribution of bla NDM among specimens were 25(56%) isolates from pus, 10(22.2%) isolates from urine, 2(4.44%) isolates from blood 2(4.44%) isolates from sputum ,one from bronchial wash(2.22%),one from drain fluid (2.22%),one (2.22%)from biopsy specimen,one (2.22%)from wound swab and one from catheter tip(2.22%). This study indicated that the incidence of pyogenic infection is expected to be highest and was referred from surgical disciplines. Similar to the study report of **Wankhede e**t al, stated that Pus (37.29%) was a most predominant specimen in his study.

6.5 Distribution of blaNDM-1 isolates among wards:

Out of 45 blaNDM-1 positive isolates majority of them 27 isolates(60%) distributed in surgery wards followed by 4 isolates(8.9%) from urology ward ,2 isolates (4.4%) each from SNN,OG and Medicine ward ,1 isolate(4.4%) each from PICU, ISCU, IMCU , paediatric ward , thoracic medicine and from casualty ward.

Wankhede et al reported that distribution of organisms in different wards (N=59) were Medicine(n=31),OG(N=13),Surgery(n=8), cardiac surgery(n=1), Paediatric ward(n=1),Ortho(N=4) ,ENT(n=1)

6.6 Risk factors:

In this study group all the isolates were nosocomial isolates. Out of 45 blaNDM - 1 positive isolates 28(62.2%) patients were admitted in the hospital for more than 2 weeks,26 (57.8%) were diabetic patients and 23 positive patients were exposed to carbapenams.

Associated risk factors for the carbapenemase production among *Enterobacteriaceae* includes certain situations, such as being transferred between hospitals or from long-term care centers, ICU admission, the presence of a central venous catheter, usage of antibiotics, and having diabetes mellitus [15, 16].

Arindam Ckakraborty et.al *study* found a significant association ($P < 0.05$) between diabetics and infection with *bla*NDM-1 producers.

6.7 Detection methods:

Out of 2514 samples screened during the study period 425 samples were *Enterobacteriaceae* (16.9%) of which 195 samples were MDR *Enterobacteriaceae* (45.89%).

6.7.1 Antibiotic susceptibility testing:

All isolates(100%) were resistant to Cefotaxime and Ceftazidime ,183 (93.8%) were resistant to ceftriaxone 186 (95.4%) were resistant to Amikacin,184(94.3%) were resistant to Cotrimoxazole,181 (92.8%)were resistant to Ciprofloxacin,48 (24.61%) were resistant to Ertapenem,39 isolates (20%) were resistant to Piperacillin /Tazobactam,Tigecycline was resistant to 6 isolates (3.07%),Colistin was resistant to 26 isolates(13.33%) and all isolates were sensitive to Aztreonam. Similar antibiotic susceptibility profiling was reported by **Ramesh et al.**

Moellering RC et.al described that presence of other accompanying genes along with bla NDM -1 for the development of multidrug resistance[29]

6.7.2 Detection **of carbapenamase producers:**

Out of 195 multi drug resistant isolates 48 (24.8%) isolates showed resistance to ertapenem. MIC was determined by Ertapenam E-test. Out of this 45 (93.75%) were positive for blaNDM PCR.

Nordmann *et al* recommended that Ertapenem as the most appropriate carbapenem for detecting NDM-1 producers[18]. Similar report was also found in study of **Parveen** et,al that 20.3% of isolates resistant to Ertapenem among Klebsiella species.

6.7.3. Detection of metallobeta lactamses by phenotypic methods:

Modified hodge test was positive for 32 isolates out of which 31 isolates were positive for bla NDM PCR. .Sensitivity of the test was 68% and specificity was 66.7%

.

Similar study by **Tzouvelekis et al** shows that the modified Hodge test had a sensitivity and specificity and of 61% and 93% respectively.[106].

Edelstein M, et al in his study reported that, MHT only screens for a phenotype, and hence with this method we cannot distinguish between the various carbapenemases producers. MBLs in particular can be difficult to detect using these enzymatic assays. Because of these drawbacks detection of these organisms pose unique challenges in the laboratory[31] Similar report by **Bashir** *et al* showed that MHT was positive in 55 of the 93 meropenem resistant isolates . **T Rimrang** B et al reported that more than 50% of the NDM-1 positive *Enterobacteriaceae* isolates were positive for Hodge test compared to KPC-producing strains[111.] **Arif Sultan** et.al reported in 2013 that

sensitivity , specificity, positive predictive value and negative predictive value for Modified Hodge Test are 42.8%, 69.8% , 94.2% and 99.6% respectively. [108]

CDT detected 32 out of 45 bla NDM PCR positive isolates with a sensitivity and specificity of about 71.1% and 66.7%. The sensitivity is relatively similar to the study of **Doyle et, al**, in his study he reported the sensitivity of 78% and specificity is high about 93%.[104]

In our study, MBL E test detected 36 out of 45 isolates with a sensitivity and specificity of about 76.5% and 100% respectively. Specificity is similar to the report of **Doyle** et al, reported that sensitivity was 78% when using the MBL Etest and the specificity was 100%[104]

Franklin et.al described that MBL - Etest using imipenem and imipenem-EDTA combination was a dependable test for the detection of enzymatic activity of MBL.[116.]

Kaleem F, et al in a study reported that the MBL E test can be used to confirm the MBL[117] In our study Carbapenemase inactivation test detected 45(93.75%) of 48 isolates of which 42 were positive for bla NDM PCR other three isolates may represent other carbapenemase genes with a sensitivity of about 93.3% and specificity 33.3%. Zwaluw et al in his study found a similar report and reported that Carbapenem inactivation test is a highly sensitive and cost effective screening method and shows high concordance with PCR, also reported positive and negative predictive value was 100% for *Enterobacteriaceae* that is similar to our study report.

Nordmann *et,al* reported that the main limitation of these phenotypic assays are failure of detecting positive isolates with low level antibiotic resistance so give a false negative report. [114]

6.7.4 **Detection by genotypic methods:**

In our study out of 48 carbapenemase resistant *Enterobacteriaceae* 45 (93.75%) were positive for blaNDM-1 by multiplex PCR. Nordmann et, al in 2009 described that phenotypic tests are unsuccessful in identification of isolates carrying several genes encoding carbepenemase production, which were effectively assessed only by genotypic methods. Phenotypic methods are growth dependent and time-consuming as it takes 18 – 24 hours to completion, not clinically useful. Results for these phenotypic assays are also subjective.[6]

Therefore, identification by molecular methods such as real time-PCR have been shown to be sensitive and more accurate for identification for detection of carbapenemase but sometimes may miss unusual gene types and require trained personnel and costlier equipments. For the identification of blaNDM-1 gene, Polymerase chain reaction has been used principally in research laboratories and reference centers.

In the present study carbapenemase inactivation test is highly sensitive (99.3%) compared to other tests. Hence we can use this test as a screening tool but the specificity is very low because of the presence of other carbapenemase encoding genes but MBL E test is a highly specific test we can use this test for screening of MBL in the absence of PCR.

Shenoy *et.al* described that though phenotypic assays are specific, do not differentiate between chromosomal and plasmid encoded genes therefore

genotypic characterization should be considered[67]

Diana Doyle *et, al* reported that sensitivity and specificity of PCR is 100% sensitivity and specificity.[119]

7. SUMMARY

This study was undertaken at Department of Microbiology, Tirunelveli Medical College, Tirunelveli for a period of 8 months from December 2015 August 2016 .Study group includes 195 MDR *Enterobacteriaceae* clinical isolates. These isolates were evaluated for carbapenem resistance by Kirby Bauer disc diffusion method with ertapenem disc 10µg and by Ertapenem E-test. MBL detection was phenotypically done by Modified Hodge test, combined disk test, MBL E-test and carbapenemase inactivation test. The presence of blaNDM-1 gene among the Carbapenem resiatant isolates were identified by multiplex PCR. Risk factors for acquisition of blaNDM-1 were also analyzed.

- Out of total of 195 isolates of Multidrug resistant Enterobacteriaceae, 131(67%) were from males and the remaining 64(33%) were from females. Mean age was 45.63years.
- Kirby Bauer disc diffusion test using Ertapenem disc and Ertapenem E-test detected 48 CRE isolates by measuring the zone size of ≤ 18 mm in diameter and MIC ≤ 4.
- Multiplex PCR detected bla NDM-1 gene in 45(93.75%) of the 48 CRE isolates.
- MHT detected 32 (64.58%) out of 45 blaNDM-1 positive isolates.
- CDT detected 32 (68.75%) out of 45 blaNDM-1 positive isolates.
- MBL E-test detected 36(75%) out of 45 bla NDM isolates.
- Carbapenemase inactivation test detected 42(93.3%) out of 45 blaNDM-1 positive isolates.

- Out of 45 blaNDM-1 positive isolates 32 (71%) blaNDM-1 positive isolates were from males and 13 (29%)were from females.
- Mean age of the blaNDM-1 isolates 45.73 years and male to female ratio was 2.4:1.
- Surgery ward accounted for 27 (70%) of the 45 blaNDM positive isolates while each two (4.4%%) from SNN,Nephrology ward ,OG and Medicine and each one from PICU (2.2%),ISCU, IMCU, Pediatric ward and casualty ward .
- Majority of the NDM infections were associated with wound infection in 27 isolates (60%) followed by UTI in 10 isolates (22.2%) and Sepsis in 5 (11.1%) isolates, Pulmonary infection in 3 isolates (6.38%)
- Duration of stay at hospital for more than two weeks had statistically significant association with the infection due to blaNDM isolates.
- Exposure to carbapenems was statistically significant among corresponding CRE patients.
- BlaNDM positive isolates showed 100% resistance to Ceftazidine, cefotaxime, ceftazidime-clavulanic acid and ertapenem, 93.3% resistance to ceftrixone, 88.9 % of isolates were resistant to amikacin and cotrimoxazole 93.3% resistance to ceftriaxone 86.7% resistance to ciprofloxacin. These isolates were 95.5% sensitive to Tigecycline and 84.5% sensitive to colistin.

8. CONCLUSION

- This study highlights the prevalence of CRE among clinical samples and also bla NDM among CRE. Antibiogram of carbapenem sensitive and resistant isolates differs and susceptibility testing is mandatory for clinical isolates of *Enterbacteriaceae* before initiation of treatment as few antibiotics are available for infection due to this superbugs.

- Kirby bauer disc diffusion method is easy to perform and cost effective in detection of carbapenamase resistance.

- Carbapenamase inactivation test is to be considered as a diagnostic tool for MBL detection because of its rapidity,cost effectiveness and more sensitivity as well as highly concordant with blaNDM PCR.

- Genotypic methods are essential for detection of blaNDM-1 gene to prevent the dissemination of these pathogens.
- These detection methods have a vital role in recognizing these organisms as epidemiologically important pathogens ,Quantifying the enormity of CRE within the facility and regionally, identifying colonized and infected patients when present in healthcare amenities and implementing intervention strategies includes to establishment of awareness with health care workers and upholding of good hygienic practices and successful barrier precautions are to be followed to prevent further transmission.

ANNEXURE – 1

1. Nutrient agar medium:

Composition

Ingredients gram/liter

Peptic digest of Animal Tissue 5.00

Sodium Chloride 5.00

Beef Extract 1.50

Yeast Extract 1.50

Agar 15.00

Twenty-eight grams of dehydrated nutrient agar medium was added to 1000 ml of cold distilled water in a flask and boiled to dissolve the medium completely. The medium was then sterilized in an autoclave at 121^0C and 15 lbs pressure for 15 minutes. The sterile media were stored in a refrigerator at 4^0C for future use.

2. MacConkey agar medium:

Composition - Ingredients gram/liter

Peptone 19.0

Lactose 10.0

NaCl 5.0

Na- Deoxycholate 1.0

Neutral Red 0.03

Crystal Violet 0.001

Agar 15.0

Fifty-two grams of dehydrated MacConkey agar medium was suspended in 1000 ml of cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121^0C and 15 lbs pressure for 15 minutes.

3. Blood agar medium

Composition

Ingredients gram/liter

Heart infusion 500.00

Tryptose 10.00

Sodium chloride 5.00

Agar 15.00

Forty grams of the dehydrated blood agar medium was suspended in 1000 ml cold distilled water in a flask and boiled to dissolve the medium completely. It was then sterilized by autoclaving at 121^0C and 15 lbs pressure for 15 minutes. The autoclaved materials were allowed to cool to a temperature of 45^0C in a water bath. Defibrinated 5-10% sheep blood was then added to the medium aseptically and distributed to sterile petri dishes. Sterile media was stored in refrigerator at 4^0C for future use.

4. Muller Hinton agar medium

Composition

Ingredients gram/liter

Beef dehytrated infusion 300

Casein hydrolysate 17.50

Starch agar 17.00

Agar 17.00

Thirty-eight grams of dehydrated Mueller Hinton agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121^0C and 15 lbs pressure for 15 minutes. The autoclaved media was stored in the refrigerator and used later.

5. McFarland Standard (0.5):

Reagents:

Sulphuric acid, 1%: To 100 ml of distilled water,1 ml of conc.sulphuric acid is added. Barium chloride, 1.175%: To 100 ml of distilled water, 1.175gm of barium chloride is added and mixed well.

To prepare McFarland 0.5 standards:

To 85 ml of 1% conc.sulphuric acid, 0.5 ml of Barium chloride is added in a flask while constantly swirling the flask. Bring to 100 ml with 1% conc.sulphuric acid. Aliquot in test tubes and cap tubes tightly. Store in the dark at room temperature for 3 months or longer.

ANNEXURE-II

PROFORMA

Name :

Age :

Sex :

OP/IP No :

Lab No :

Ward :

Complaints :

Clinical diagnosis :

Nature of Specimen :

Duration of hospital stay :

Antibiotics administered :

Investigation :

Biochemical tests : Indole, Citrate, Urease, Triple sugar iron, Catalase, Oxidase

Antibiogram :

Piperacillin, Amikacin, Ceftriaxone, Cefotaxime, Ceftazidime, Ciprofloxacin, Ofloxacin, Gentamicin, Ertapenam, Piperacillin with Tazobactam, Ceftazidime with clavulanic acid, Colistin. Tigecycline

Screening test with Ertapenem

1.Disc diffusion test

2.E-test

Screenining test for metallo beta lactamase production:

1.Combined disc test

2.MHT

3.MBL E-test and

4.Carbepenemase inactivation test)

RT- PCR

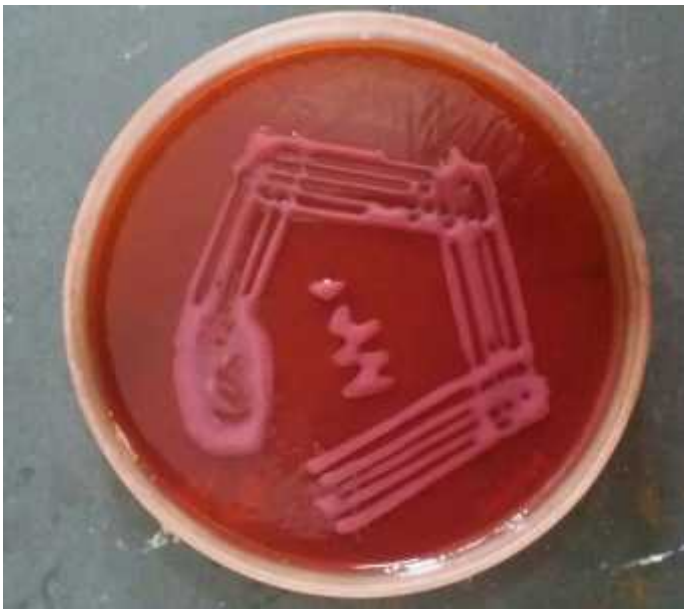
COLOUR PLATE:1

A. MAC CONKEY AGAR PLATE SHOWING LACTOSE FERMENTING COLONIES OF E.coli

B.NUTRIENT AGAR PLATE SHOWING MUCOLI COLONIES OF KLEBSIELLA SPECIES

COLOUR PLATE ;2

A. MAC CONKEY AGAR PLATE SHOWING MUCOID LACTOSE FERMENTING COLONIES OF KIEBSIELLA SPECIES



B.NULTRIENT AGAR PLATE SHOWING SWAMING OF PROTEUS

COLOUR PLATE:3

A.DISC DIFFUSION TEST: showing Ertapenem sensitivity

B.DISC DIFFUSION TEST: showing Ertapenem resistance

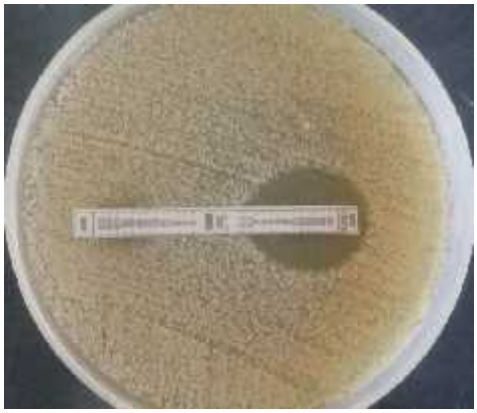
COLOUR PLATE:4

A.ERTAPENEM E-TEST: showing Ertapenem resistance

B.COMBINED DISK DIFFUSION TEST: showing MBL production

COLOUR PLATE:5

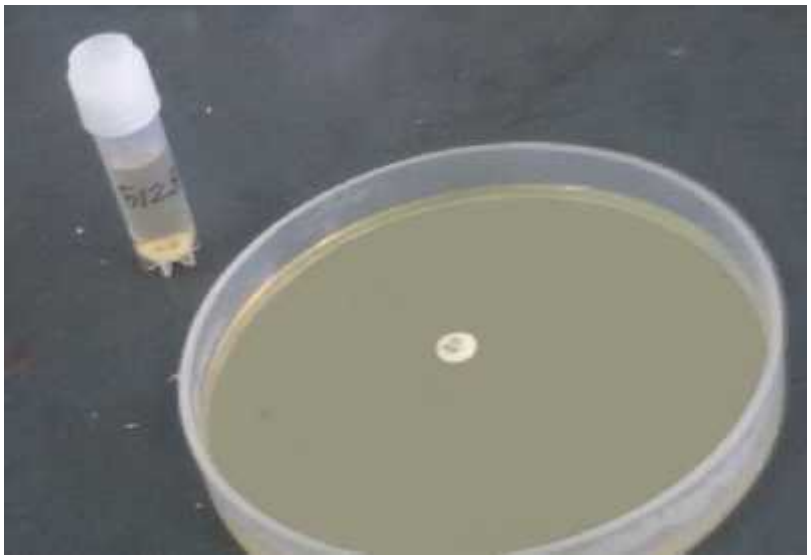
A.MBL E-Test: showing MBL production



B.MODIFIED HODGE TEST:

COLOUR PLATE:6

CARBAPENEMASE INACTIVATION TEST: showing MBL production



After Incubation

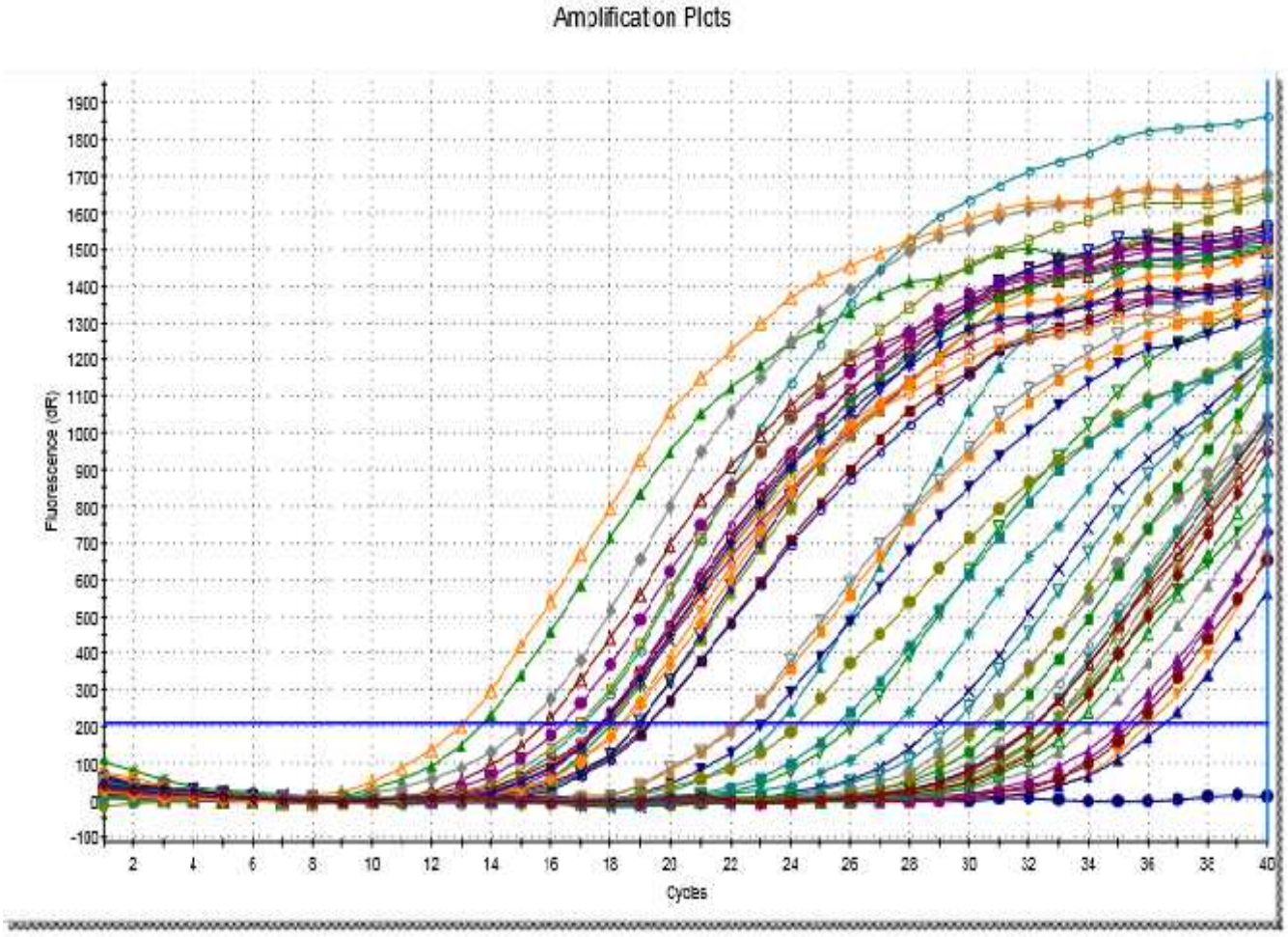
COLOUR PLATE:7

A.THERMOCYCLER:

B.DNA EXTRACTION KIT:

COLOUR PLATE:8

REAL TIME PCR AMPLIFICATION CHART:



MASTER CHART

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime /Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation tes	PCR
												SE	AST pattern by agar diffusion																			
1	##	0/36	m	SNN-3	BLO	*K.pneum*	SEPSIS	Preterm. LBW	0 day	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	R	_	_	_	-	-	-
2	##	3/36	M	SNN-3	BLO	*K.pneum*	SEPSIS	Preterm. LBW	13day	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	R	_	_	_	-	-	-
3	##	7/365	m	SNN-3	BLO	*K.pneum*	SEPSIS	Preterm. LBW	7days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	_	_	_	-	-	-
4	##	3/365	F	SNN-1	BLO	*K.pneum*	SEPSIS	Preterm. LBW	DAY	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	_	_	_			-
5	##	5/365	M	SNN-3	BLO	*K.pneum*	SEPSIS	Preterm. LBW	DAY	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	_	_	_	-	-	-
6	##	4/365	F	SNN-1	BLO	*K.pneum*	SEPSIS	Preterm. LBW	4 days	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	R	_	_	_	_	_	_
7	##	6/365	F	SNN-3	BLO	*K.pneum*	SEPSIS	Preterm. LBW	6-days	y	R	R	R	R	R	R	R	R	R	S	R	S	s	R	R	p	P	p	p	P	P
8	##	3/365	M	SNN-3	BLO	*K.pneum*	SEPSIS	Preterm. LBW	8days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	p	N	N	N	p	N
9	##	4/365	M	SNN-2	BLO	*K.pneum*	SEPSIS	Preterm. LBW	7days	y	R	R	R	R	R	R	R	R	R	S	R	S	s	R	R	P	N	N	N	p	N
#	##	7/365	F	SNN--3	BLO	*K.pneum*	SEPSIS	Preterm. LBW	8 Day	y	R	R	R	R	R	R	R	R	R	S	R	S	S	R	S	P	P	p	P	N	P
#	##	0/36	F	SNN--2	BLO	*K.pneum*	SEPSIS	Preterm. LBW	0 day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	_	_	_	_	_	_
#	##	9/365	M	SNN-2	BLO	*K.oxytoc*	SEPSIS	Preterm. LBW	9days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	_	_	_	_	_	_
#	##	Mont	F	PICU	BLO	*K.pneum*	SEPSIS	Preterm. LBW	8 days	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	R	_	_	_	_	_	_
#	##	6/365	F	SNN--2	BLO	*K.pneum*	SEPSIS	Preterm. LBW	6 days	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	R	_	_	_	_	_	—
#	##	7/365	F	SNN-2	BLO	*K.pneum*	SEPSIS	Preterm. LBW	7 days	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	R	_	_	_	_	_	_
#	##	62	F	S-2	UR	*E.coli*	UTI	POST SX	5 Day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_
#	##	58	M	S-2	UR	*E.coli*	UTI	POST SX	5 day	N	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	P	P	p	p	P	P
#	##	70	M	M-2	UR	*E.coli*	UTI-CA	CA	5 Day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_
#	##	2	M	PICU	UR	*E.coli*	UTI	SURGERY	6days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified Hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation test	PCR	
							DI					SE	AST pattern by agar diffusion																			
#	##	78	M	M-2	UR	*E.coli*	UTI-CA	CA	4-Day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	65	M	S-2	UR	*E.coli*	UTI-CA	CA	5-days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	34	M	M-OP	UR	*E.coli*	UTI	NIL	NIL	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	70	M	M-4	UR	*E.coli*	UTI	CA	6Days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	75	M	S-6	UR	*E.coli*	UTI	POSTS URGERY	8days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S		-			
#	##	10	F	PAE.W	UR	*E.coli*	UTI	MALNUITRITION	2days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	36	F	IMCU	UR	*E.coli*	UTI	NIL	5days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	56	M	S-4	UR	*E.coli*	UTI	POSTS URGERY	6 days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	27	F	OG-2	UR	*E.coli*	UTI	POSTS URGERY	2days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	3	F	P.W	UR	*K.oxytoc*	UTI	URETERIC REFLEX	3 Day	N	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	P	N	P	P	P	P
#	##	Mon	M	P.Sur	UR	*E.coli*	UTI	POST SURGERY	2days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	23	F	S-5	UR	*E.coli*	UTI	NIL	4days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	18	M	Uro.W	UR	*K.oxytoc*	UTI	URETERIC STRICTUER	5days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	65	M	Uro.W	UR	*K.oxytoc*	UTI	POST SURGERY	5days	Y	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	65	M	Uro.W	UR	*K.oxytoc*	UTI	POST SURGERY	6Days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					
#	##	10	M	M.OP	RIN	*E.coli*	UTI	NIL	NIL	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	AST pattern by agar diffusion: Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation test	PCR	
#	##	Mont	M	MCH	UR	*E.coli*	UTI	POST SURGERY	7days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	75	M	IMCU	UR	*E.coli*	SEPSIS	DM	5days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	Mont	M	P.Sur	UR	*E.coli*	UTI	POST SURGERY	3Days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	3	M	P.Sur	UR	*E.coli*	UTI	POST SURGERY	2days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	-	_	
#	##	60	F	URO-2	UR	*K.oxytoc*	URETERI	POSTS URGERY	7days	N	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	_	_	_	_	_	_	
#	##	3	M	PICU	UR	*E.coli*	UTI	POST SURGERY	4days	N	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	_	_	_	_	_	_	
#	##	55	M	S-2	UR	*E.coli*	UTI	POST SURGERY	8days	Y	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	_	_	_	_	_	_	
#	##	74	M	S-1	UR	*E.coli*	UTI-CA	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	_	_	_	_	_	_	
#	##	72	F	URO OP	UR	*E.coli*	UTI-CA	CATHETER	NIL	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	74	M	IMCU	UR	*E.coli*	UTI	DM	6Days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	3	F	FCH	UR	*E.coli*	UTI	URETERIC REFLEX	2days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	47	M	S-1	UR	*K.pneum*	UTI	POST SURGERY	5days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	74	M	IMCU	UR	*E.coli*	SEPSIS	DM	4days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	64	M	M-4	UR	*E.coli*	UTI	DM	3Days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	55	M	M-2	UR	*E.coli*	UTI	DM	4days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_	
#	##	55	F	S-2	PUS	*K.pneum*	Non healn	DM	6 day	Y	R	R	R	R	R	R	R	R	R	S	R	S	S	R	S	P	P	p	p	P	P	
#	##	45	M	S-2	PUS	*P.vulgari*	Non healn	DM	19day	Y	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	R	P	p	p	P	P	
#	##	55	M	CAS.W	PUS	*P.mirabi*	Non healn	DM	0 Day	Y	R	R	R	R	R	R	R	R	s	S	R	S	S	R	R	R	P	N	N	P	P	P

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	AST pattern by agar diffusion																								Carbapenamase inactivation tes	
											Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Meropenem E-test	Combined disc diffusion test	MBL E-test	PCR		
#	##	35	M	NEP.W	CA	*K.pneum*	SEPSIS	CKD	5 Day	N	R	R	R	R	R	R	R	R	S	R	S	R	S	P	P	p	p	P	P		
#	##	45	M	S-7	DR	*K.oxytoc*	SSI	POST SURGERY	3days	N	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	_	_	_	_	_		
#	##	55	M	S-2	PUS	*K.oxytoc*	Non healn	DM	1 day	Y	R	R	R	R	R	R	R	R	S	R	S	R	R	P	P	p	P	P	P		
#	##	59	F	PUL.W	BR(	*E.coli*	Pneumonia	CHRONIC LUNG DISEASE	2 Day	N	R	R	R	R	R	R	R	R	S	R	S	R	S	P	P	p	p	P	P		
#	##	50	F	FS-1	PUS	*P.vulgari*	Non healn	DM	8 Day	Y	R	R	R	R	R	R	R	R	S	R	S	R	R	P	P	p	p	P	P		
#	##	65	M	S-2	PUS	*K.oxytoc*	Non healn	DM	7 Day	Y	R	R	R	R	R	R	R	R	S	R	S	S	R	S	P	N	P	P	p	p		
#	##	60	M	S-2	PUS	*K.oxytoc*	Non healn	DM	6 Day	Y	R	R	R	R	R	R	R	R	S	R	R	S	R	S	P	P	p	p	P	P		
#	##	65	M	M-4	PUS	*E.coli*	Abscess	DM	7days	N	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	P	N	p	P	P		
#	##	29	F	OG-2	pus	*K.pneum*	Wound inf	POST SURGERY	9 Days	N	R	R	R	R	R	R	R	R	S	R	S	S	R	S	P	N	N	N	P	P		
#	##	10	M	PAED.SX	Wo	*K.pneum*	Wound inf	POST SURGERY	8days	N	R	R	R	R	R	R	R	S	R	S	R	S	S	R	S	P	N	N	N	P	P		
#	##	59	M	M-4	Spu	*K.pneum*	Pneumonia	DM	5 day	Y	R	R	R	R	R	R	R	R	S	R	S	S	R	S	P	P	p	P	P	P		
#	##	55	F	S-2	PUS	*K.pneum*	Non healn	DM	10day	Y	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	P	p	P	P	P		
#	##	60	M	S-2	pus	*P.mirabi*	Non healn	DM	8days	N	R	R	R	R	R	R	R	S	R	S	S	S	R	R	S	_	_	_	_	_	_		
#	##	35	M	NEP.W	Cat	*K.pneum*	SEPSIS	CKD	8 day	N	R	R	R	S	R	R	R	R	R	S	R	S	R	R	R	P	P	p	P	P	P		
#	##	65	M	S-3	pus	*K.pneum*	Wound inf	POST SURGERY	7days	N	R	R	R	R	R	R	R	R	S	S	S	S	R	R	R	_	_	_	_	_	_		
#	##	51	M	IMCU	pus	*K.oxytoc*	CELLULI	DM	6 day	Y	R	R	R	R	R	R	R	R	S	R	S	R	R	S	P	P	p	N	P	P		
#	##	60	M	M-4	pus	*K.oxytoc*	Non healn	DM	8days	N	R	R	R	R	R	R	R	S	S	S	S	S	R	S	_	_	_	_	_	_		
#	##	65	F	S-4	pus	*P.vulgari*	Non healn	DM	8days	N	R	R	R	R	R	R	R	S	S	S	S	R	R	S	P	_	_	_	_	_		
#	##	55	M	ORTHO-3	pus	*P.vulgari*	Wound inf	DM	9days	Y	R	R	R	R	R	R	R	R	S	S	S	R	R	S	_	_	_	_	_	_		
#	##	45	F	S-2	puS	*P.vulgari*	Non healn	DM	10day	Y	R	R	R	R	R	R	R	R	S	R	S	R	R	R	P	N	N	P	N	P		
#	##	55	F	S-7	PUS	*K.oxytoc*	Non healn	DM	11day	Y	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	N	N	N	P	P		
#	##	19	F	OG-2	PUS	*K.oxytoc*	Non healn	DM	10day	N	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_				_	_		
#	##	52	M	ISCU	PUS	*K.oxytoc*	Wound inf	POST SURGERY	6 day	N	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	P	p	P	P	P		

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation tes	PCR
#	##	30	F	ORTHO-3	PUS	*K.oxytoc*	Wound inf	POST SURGERY	9days	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	_				_	_
#	##	60	M	THOR.M	PUS	*K.oxytoc*	LUNG ABSE	CHRONIC LUNG DISEASE	7 Day	Y	R	R	R	S	R	R	R	R	R	S	R	S	S	R	R	P	P	p	P	P	P
#	##	46	M	THOR.M	Spu	*K.oxytoc*	Pneumonia	DM	12day	N	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S					_	_
#	##	27	F	LW.ICU	PUS	*K.pneum*	Wound inf	POST SURGERY	8days	N	R	R	R	S	R	R	R	R	R	S	S	S	S	R	S					_	-
#	##	30	M	BURNS .W	PUS	*K.oxytoc*	BURNS	BURNS	8days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	63	M	S-2	PUS	*K.pneum*	Non healIn	DM	8days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	53	M	CAS.W	PUS	*P.mirabi*	Abscess	DM	8days	N	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S					_	_
#	##	47	M	CAS.W	PUS	*P.mirabi*	Abscess	DM	7days	Y	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S					_	_
#	##	49	F	S-5	PUS	*P.vulgari*	Wound inf	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S					_	_
#	##	50	F	S-2	PUS	*P.vulgari*	Wound inf	POST SURGERY	12days	N	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S					-	_
#	##	62	M	S-OP	PUS	*P.mirabi*	Non healIn	DM	11day	Y	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S					_	_
#	##	50	M	S-3	PUS	*K.oxytoc*	Non healIn	DM	7 DAY	N	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	P	p	P	P	p
#	##	38	M	S-3	PUS	*E.coli*	Abscess	NIL	11day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	46	M	S-3	PUS	*E.coli*	Abscess	DM	12day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	44	M	S-7	PUS	*K.oxytoc*	CELLULIT	DM	8 day	Y	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	N	N	P	N	P
#	##	50	F	S-4	PUS	*P.vulgari*	Abscess	DM	11day	N	R	R	R	R	R	S	R	R	R	S	R	S	R	R	R	P	N	N	N	P	P
#	##	54	M	CAS.W	PUS	*K.oxytoc*	Abscess	DM	10day	Y	R	R	R	S	R	R	R	R	R	S	R	S	S	R	S	P	P	p	N	N	N
#	##	55	M	S-1	PUS	*K.oxytoc*	Abscess	DM	6 day	Y	R	R	R	S	R	R	R	R	R	S	R	S	S	R	R	P	P	p	N	P	P
#	##	45	M	S-5	PUS	*K.oxytoc*	Abscess	DM	6 day	N	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	P	p	P	P	P
#	##	61	M	S-2	PUS	*K.oxytoc*	Abscess	DM	8days	Y	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	-				-	_
#	##	60	F	M-4	SPU	*K.pneum*	Pneumonia	CHRONIC LUNG DISEASE	12day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	-				_	_

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified Hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation tes	PCR	
#	##	55	F	S-4	PUS	*K.oxytoc*	Abscess	DM	11day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	-				_	_
#	##	11	M	P.W	PUS	*K.oxytoc*	Abscess	MALNUITRITION	10day	N	R	R	R	S	R	R	R	R	R	S	S	S	S	R	S	_				_	_
#	##	37	M	S-7	PUS	*K.pneum*	CELLULI	DM	9days	N	R	R	R	S	R	R	S	R	R	S	S	S	S	R	S					_	_
#	##	42	F	SKIN OP	PUS	*E.coli*	Abscess	DM	9days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	55	M	S-1	PUS	*E.coli*	Abscess	POST SURGERY	10day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	40	M	CAS.W	PUS	*K.pneum*	Abscess	DM	10day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	54	M	M-6	Spu	*K.oxytoc*	Pneumonia	CHRONIC LUNG DISEASE	9days	N	R	R	R	S	R	R	S	R	R	S	S	S	S	R	S					_	_
#	##	68	M	M-4	PUS	*K.pneum*	Abscess	DM	9days	Y	R	R	R	R	R	S	R	R	R	S	S	S	S	R	S					_	_
#	##	41	M	S-4	PUS	*P.vulgari*	Abscess	POST SURGERY	9days	N	R	R	R	R	R	S	R	R	R	S	S	S	R	R	S					_	_
#	##	55	M	OG-2	PUS	*E.coli*	Abscess	POST SURGERY	10day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	24	F	FS-2	PUS	*P.vulgari*	Abscess	NIL	11day	Y	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S					_	_
#	##	64	M	S-6	PUS	*P.vulgari*	Abscess	POST SURGERY	9days	Y	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S					_	_
#	##	43	F	OG-2	PUS	*K.oxytoc*	SSI	POST SURGERY	8 day	N	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	N	N	N	P	N
#	##	26	F	S-2	PUS	*K.oxytoc*	Abscess	DM	11day	N	R	R	R	R	R	S	R	S	R	S	S	S	S	R	S					_	_
#	##	65	M	S-2	PUS	*K.oxytoc*	Abscess	DM	8 day	N	R	R	R	R	R	R	R	R	R	S	R	S	S	R	S	P	N	N	N	P	P
#	##	60	M	M-4	PUS	*K.pneum*	Abscess	DM	9days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_
#	##	65	M	S-4	Spu	*K.oxytoc*	Pneumonia	CHRONIC LUNG DISEASE	6 day	Y	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	P	p	P	P	P
#	##	65	M	S-3	PUS	*K.pneum*	Abscess	DM	10day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					_	_

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation tes	PCR	
#	##	51	M	ISCU	pus	*K.oxytoc*	Non healI	DM	11day	N	R	R	R	R	R	R	R	R	R	S	S	R	R	R	S	_				_	_
#	##	59	M	M-1	Spu	*K.oxytoc*	Pneumonia	CHRONIC LUNG DISEASE	9days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					-	_
#	##	80	M	IMCU	CSI	*K.pneum*	Meningitis	DM	11day	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_				-	_
#	##	25	M	URO-1	UR	*K.pneum*	URETERI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					-	_
#	##	60	F	OG-2	VA	*K.oxytoc*	Cervicitis	POST MENOPAUSAL	8days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					-	_
#	##	10	F	PAED WD	Wo	*K.oxytoc*	Abscess	MALNUITRITION	8days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S					-	_
#	##	10	M	M.OP	UR	*E.coli*	UTI	NIL	7days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	_
#	##	Mont	M	PAE.SX	UR	*E.coli*	UTI	POST SURGERY	10day	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	_
#	##	70	M	S-3	UR	*E.coli*	BPH	POST SURGERY	11day	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	_
#	##	45	M	URO-2	UR	*E.coli*	UTI	POST SX	12day	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	_
#	##	3	M	PICU	UR	*E.coli*	UTI	POST SURGERY	9days	Y	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	_
#	##	60	F	S-2	UR	*K.oxytoc*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	_
#	##	3	M	S-1	UR	*E.coli*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	55	M	URO-2	UR	*E.coli*	UTI	POST SURGERY	8days	N	R	R	R	R	R	R	R	S	NA	S	S	S	S	R	S					_	_

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation test	PCR
#	##	74	M	S-1	UR	*E.coli*	UTI	POST SURGERY	10days	N	R	R	R	R	R	R	R	S	NA	S	S	S	S	R	S					-	_
#	##	72	M	URO-2	UR	*E.coli*	UTI	BPH	10days	N	R	R	R	R	R	R	R	S	NA	S	S	S	S	R	S					_	_
#	##	69	F	URO	UR	*E.coli*	URETERI	BPH	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	24	M	URO-2	UR	*E.coli*	URETERI	NIL	9days	N	R	R	R	R	R	R	S	R	NA	S	S	S	S	R	S					_	_
#	##	7	F	PICU	UR	*E.coli*	UTI	POST SURGERY	8days	N	R	R	R	R	R	S	R	R	NA	S	S	S	S	R	S					_	_
#	##	25	M	S-2	UR	*E.coli*	UTI	NIL	10days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	45	M	URO-2	UR	*K.oxytoc*	URETERI	POST SURGERY	11days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	57	M	S-1	UR	*K.oxytoc*	UTI	POST SURGERY	12days	Y	R	R	R	R	R	R	S	R	NA	S	S	S	S	R	S					_	_
#	##	62	M	URO-2	UR	*K.oxytoc*	BPH	POST SURGERY	9days	N	R	R	R	R	R	R	S	R	NA	S	S	S	S	R	S					_	_
#	##	45	M	S-1	UR	*K.oxytoc*	URETERI	POST SURGERY	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	76	M	URO	UR	*K.oxytoc*	BPH	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	R	R	S	S	R	R	P	P	p	P	P	P
#	##	77	M	M-1	UR	*E.coli*	SEPSIS	DM	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	60	M	M-4	UR	*P.vulgari*	UTI-CA	DM	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	R	R	S					_	_
#	##	70	M	URO	UR	*K.oxytoc*	BPH	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	70	F	IMCU	UR	*E.coli*	SEPSIS	DM	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S	_				_	_
#	##	45	F	IMCU	UR	*E.coli*	SEPSIS	DM	8days	N	R	R	R	R	R	S	R	R	NA	S	S	S	S	R	S					_	_
#	##	26	F	IMCU	UR	*E.coli*	SEPSIS	NIL	11days	N	R	R	R	R	R	R	S	R	NA	S	S	S	S	R	S					_	_
#	##	26	F	OG-2	UR	*E.coli*	UTI	NIL	10days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	65	F	LW.ICU	UR	*E.coli*	UTR.PRO	POST SURGERY	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_
#	##	70	M	URO.OP	UR	*K.oxytoc*	UTI	BPH	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					_	_

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	AST pattern by agar diffusion																								Carbapenamase inactivation tes	
											Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified Hodge test	Combined disc diffusion test	MBL E-test	PCR	
#	##	23	F	OG-2	UR	*K.oxytoc*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						-	_
#	##	56	M	NEP.W	UR	*E.coli*	UTI	CKD	9days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						-	_
#	##	50	M	M-4	UR	*K.oxytoc*	UTI	CVA	8days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	30	F	M-1	UR	*K.oxytoc*	UTI	NIL	9days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	37	F	URO	UR	*K.oxytoc*	URETERI	POST SURGERY	7days	Y	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	30	F	M-5	UR	*K.oxytoc*	UTI	NIL	11day	Y	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	60	M	FM-2	UR	*K.oxytoc*	UTI	DM	9days	Y	R	R	R	R	R	R	R	R	R	NA	S	R	S	S	S	R	S	P	P	p	P	P	P
#	##	1	M	PICU	UR	*K.oxytoc*	SEPSIS	POST.URETHRAL VALVE	9days	N	R	R	R	R	R	R	R	R	R	NA	S	R	S	S	S	R	S	P	P	p	P	N	P
#	##	56	M	M-5	UR	*E.coli*	UTI	DM	9days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	45	M	URO-2	UR	*K.oxytoc*	URETERI	POST SURGERY	8days	Y	R	R	R	R	R	R	R	R	R	NA	S	R	S	S	S	R	S	P	N	N	N	P	P
#	##	35	M	URO	UR	*K.oxytoc*	URETERI	POST SURGERY	11days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S	_					_	_
#	##	24	M	M-4	UR	*E.coli*	UTI	NIL	8days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S	_					_	_
#	##	7/365	M	SNN	UR	*K.oxytoc*	SEPSIS	Preterm. LBW	7days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	50	F	URO-2	UR	*E.coli*	URETERI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S	_					_	_
#	##	60	M	S-4	UR	*E.coli*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	48	F	IMCU	UR	*E.coli*	SEPSIS	DM	11day	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	74	M	URO-2	UR	*K.oxytoc*	UTI	BPH	11day	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_
#	##	65	F	URO-2	UR	*K.oxytoc*	URETERI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	R	NA	S	S	S	S	S	R	S						_	_

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified hodge test	Combined disc diffusion test	MBL E-test	Carbapenamase inactivation tes	PCR
							DI					SE	AST pattern by agar diffusion																			
#	##	42	M	S-6	UR	*E.coli*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	55	M	URO	UR	*E.coli*	URETERI	POST SURGERY	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	62	M	M-5	UR	*E.coli*	UTI	CVA	11day	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	mon	M	PAED.SX	UR	*K.oxytoc*	UTI	POST URETHAL VALVE	10day	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	32	F	OG-2	UR	*P.vulgari*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	R	R	S					-	-
#	##	40	F	M-5	UR	*E.coli*	UTI	DM	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	60	F	S-7	UR	*E.coli*	UTI	POST SURGERY	8days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	56	F	M-5	UR	*E.coli*	UTI	DM	10day	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	21	F	M-3	UR	*E.coli*	UTI	DM	10day	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	49	M	URO	UR	*E.coli*	UTI	POST SURGERY	11day	Y	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	70	F	URO	UR	*E.coli*	UTI	BPH	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	73	M	S-2	UR	*E.coli*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	55	M	M-4	UR	*E.coli*	UTI	POST SURGERY	9days	N	R	R	R	R	R	R	R	R	NA	S	S	S	S	R	S					-	-
#	##	42	M	S-4	PUS	*E.coli*	Abscess	DM	10day	Y	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	P	N	p	P	P	P
#	##	65	M	S-3	PUS	*K.pneum*	Nonhealin	DM	8 day	Y	R	R	R	R	R	S	S	S	S	S	R	S	S	R	S	P	P	p	P	P	P
#	##	50	F	S-2	bio	*P.vulgari*	Non healI	DM	5 day	N	R	R	R	R	R	S	S	S	S	S	R	S	R	R	S	P	P	P	P	P	P
#	##	65	F	S-4	PUS	*K.oxytoc*	Abscess	DM	2 day	N	R	R	R	R	R	S	S	S	S	S	R	S	S	R	S	P	N	N	N	P	P
#	##	70	M	S-2	PUS	*E.coli*	CELLULI	DM	6 day	N	R	R	R	R	R	R	R	R	S	S	R	S	R	R	S	P	N	N	P	P	P
#	##	65	M	S-4	PUS	*K.oxytoc*	CELLULI	DM	0 Day	N	R	R	R	R	R	R	R	R	S	S	R	S	s	R	S	P	P	N	P	P	P
#	##	46	F	OG-2	UR	*K.oxytoc*	UTI	Post SX	6 day	Y	R	R	R	R	R	R	S	R	NA	S	R	S	S	R	S	P	P	P	P	P	P

S.NO	MICRO NO	Age	SEX	WARD	SPECIMEN	ORGANISM	DIAGNOSIS	RISK FACTORS	HOSPITAL STAY DURATION	Prior exposure to carbapenams	AST pattern by agar diffusion																						Carbapenamase inactivation test	
											Ampicillin(30µg)	Ceftazidime(30µg)	Cefotaxime(30µg)	Ceftriaxone(30µg)	Gentamicin(10µg)	Amikacin(30µg)	Cotrimoxazole	ciprofloxacin	Doxycycline	Tigecycline	Ertapenam	Aztreonam	colistin	ceftazidime/Clavulanic acid	Piperacillin-Tazobactam	Ertapenam E-test	Modified hodge test	Combined disc diffusion test	MBL E-test	PCR	
#	##	76	M	URO	UR	*K.oxytoc*	UTI	POSTS URGERY	5 day	N	R	R	R	R	R	R	S	R	NA	S	R	S	S	R	S	P	P	P	P	P	P	P
#	##	36	M	URO-2	UR	*K.oxytoc*	URETERI	POSTS URGERY	7 day	N	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	P	P	P	P	P	P	P
#	##	70	M	U.OP	UR	*K.oxytoc*	UTI	CA	NA	Y	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	P	_				_	_
#	##	62	F	S-2	UR	*E.coli*	UTI	POST SURGERY	8 days	Y	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	P	_				_	_
#	##	56	M	Nephro	UR	*E.coli*	UTI	CKD	7days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	P					_	_
#	##	70	M	URO	UR	*E.coli*	UTI	POST SURGERY	6 days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	P	-				_	-
#	##	40	M	URO	UR	*E.coli*	UTI	POST SURGERY	6 days	N	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	_	-				_	_
#	##	56	M	S-4	UR	*E.coli*	UTI	POST SURGERY	6 day	N	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	S	P	P	P	P	P	P	P